



Analysis of titanium dioxide nanoparticles in food by ICP-MS/MS in single particle mode

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1st ISO-FOOD International Symposium on Isotopic and Other Techniques in Food Safety and Quality

Portorož, Slovenia

April 1-3, 2019



Organised by
ERA Chair ISO-FOOD in Isotope Techniques in Food Quality,
Safety and Traceability
Department of Environmental Sciences
Jožef Stefan Institute

Programme and Book of Abstracts

1st ISO-FOOD International Symposium on Isotopic
and Other Techniques in Food Safety and Quality

Portorož, Slovenia
April 1 to 3, 2019

Programme and Book of Abstracts

Organised by:

**ERA Chair ISO-FOOD in Isotope Techniques in Food Quality,
Safety and Traceability
Department of Environmental Sciences
Jožef Stefan Institute**

Edited by:

David Heath, Milena Horvat, and Nives Ogrinc

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Welcome

Dear readers and delegates, it is a pleasure for us to present you with this Book of Abstracts consisting of contributions to the first ISO-FOOD International Symposium on the use of Isotopes and Other Techniques in Food Safety and Quality. This event has been organised by the ERA Chair ISO-FOOD for isotope techniques in food quality, safety and traceability, in affiliation with the Jožef Stefan Institute in Ljubljana, Slovenia and is being held at the Grand Hotel Bernardin in Portorož, close to the historic picturesque town of Piran on the Adriatic coast.

The past few years have seen a resurgence in the need for and the development of new methods and techniques for assuring the quality, authenticity, and safety of food. Much of this need is born out of major food scandals, globalisation and the increasing complexity of the food supply chain. It also reflects changing consumer preferences such as the demand for organic and locally produced culturally authentic foods and the adoption of new technologies such as nanotechnology, which offers fascinating possibilities for improving food production, but also new analytical challenges and unknown threats. Fortunately, this has coincided with significant advances in instrumentation, where isotopic and other techniques play a key role. A need also exists to address the issue of collating, managing, and exploiting the large amounts of isotopic, elemental, nutritional, and other data (big data) that, although open and mostly digitized, has become too large and complex for traditional data management tools. To address these different topics we have divided the programme into the following six sessions: food authenticity and traceability, food safety and quality, foodomics, nanomaterials and nanotechnology, metrology in food, and food databases and semantics. We hope that this will deliver a rich and interesting programme with 15 invited speakers and 31 oral and 59 poster presentations.

Ultimately, this Symposium is about bringing together an international array of scientists and scholars, policy makers, and stakeholders to generate lively discussions and productive exchanges of ideas and experiences. We want this to be an opportunity for people to strengthen existing relationships, and foster future collaborations.

We would like to thank all of the participants in the Symposium — invited speakers, presenters, moderators, and sponsors. We would also like to thank all the members of the organising committee for their efforts in planning this event.

We hope that you enjoy the programme and have a rewarding symposium.

Symposium Organising Committee

Symposium Schedule - Sunday, March 31 to Wednesday, April 3

	Sun 3 /31	Mon 4 /1	Tue 4 /2	Wed 4 /3
8am				
		08:30 - 09:15 Opening Ceremony and Introductory Talks / <i>Europa B (12th fl.)</i>	08:30 - 09:30 Invited Lectures / <i>Europa B (12th fl.)</i>	
9am		09:15 - 09:45 Invited Lectures / <i>Europa B (12th fl.)</i>		09:00 - 09:30 Invited Lectures / <i>Europa B (12th fl.)</i>
		09:45 - 10:45 Food Authenticity and Traceability / <i>Europa B (12th fl.)</i>	09:30 - 10:30 Food Quality and Safety / <i>Europa B (12th fl.)</i>	09:30 - 10:00 Foodomics / <i>Europa B (12th fl.)</i>
10am				10:00 - 10:30 Coffee Break (Wednesday Morning) / <i>Europa D (12th fl.)</i>
		10:45 - 11:15 Coffee Break (Monday Morning) / <i>Europa D (12th fl.)</i>	10:30 - 11:00 Coffee Break (Tuesday Morning) / <i>Europa D (12th fl.)</i>	10:30 - 11:30 Invited Lectures / <i>Europa B (12th fl.)</i>
11am		11:15 - 11:45 Invited Lectures / <i>Europa B (12th fl.)</i>	11:00 - 12:00 Invited Lectures / <i>Europa B (12th fl.)</i>	
		11:45 - 12:45 Food Authenticity and Traceability / <i>Europa B (12th fl.)</i>		11:30 - 12:15 Metrology in Food / <i>Europa B (12th fl.)</i>
12pm			12:00 - 13:00 Food Quality and Safety / <i>Europa B (12th fl.)</i>	12:15 - 12:45 Invited Lectures / <i>Europa B (12th fl.)</i>
		12:45 - 14:00 Posters / <i>Europa D (12th fl.)</i>		12:45 - 13:15 Food Databases and Semantics / <i>Europa B (12th fl.)</i>
1pm			13:00 - 14:30 Lunch Break / <i>Hotel Restaurant</i>	13:15 - 13:45 Invited Lectures / <i>Europa B (12th fl.)</i>
		14:00 - 15:00 Invited Lectures / <i>Europa B (12th fl.)</i>		13:45 - Closing Ceremony / <i>Europa B (12th fl.)</i>
2pm			14:30 - 17:30 Visit to Secovlje Salt Pans / <i>Secovlje Salina Park</i>	
3pm	15:00 - 19:00 Registration / <i>Registration Desk</i>	15:00 - 15:30 Food Authenticity and Traceability / <i>Europa B (12th fl.)</i>	15:00 - 15:30 Invited Lectures / <i>Secovlje</i>	
		15:30 - 16:00 Coffee Break (Monday Afternoon) / <i>Europa D (12th fl.)</i>		
4pm		16:00 - 16:30 Invited Lectures / <i>Europa B (12th fl.)</i>		
		16:30 - 18:00 Food Quality and Safety / <i>Europa B (12th fl.)</i>		
5pm				
		18:00 - 19:30 Monday Evening Poster Session / <i>Europa D (12th fl.)</i>		
6pm				
7pm	19:00 - 20:30 Welcome Party / <i>Europa D (12th fl.)</i>		19:00 - 21:00 Dinner / <i>Hotel Restaurant</i>	
8pm				

SHORT PROGRAMME

Sunday, March 31, 2019

15:00-19:00 Registration

19:00 Welcome party

Monday, April 1, 2019

08:30-08:45 Opening ceremony

08:45-09:00 D. Heath Era Chair ISO-FOOD presentation

09:00-09:15 M. Horvat Achievements of ISO-FOOD project

FOOD AUTHENTICITY AND TRACEABILITY Moderators: **N. Ogrinc, F. Vanhaecke**

09:15-09:45 S. Kelly **A new rapid method for stable isotope analysis of non-exchangeable hydrogen to detect undeclared addition of sugar and sugar syrups to food**

09:45-10:00 L. Mounier State of the art Isotope Ratio Mass Spectrometry (IRMS) applied to food traceability

10:00-10:15 M. Ocvirk Authentication of the geographical origin of hops (*Humulus lupulus* L.)

10:15-10:30 J. Golubović Comparison of organic and conventional beer in view of the content of the selected phytoestrogens

10:30-10:45 R. Cellier Isotopic and multi-elemental signatures as indicators of origin of sparkling wines

10:45-11:15 Coffee break

FOOD AUTHENTICITY AND TRACEABILITY Moderators: **S. Kelly, K. Löschner**

11:15-11:45 N. Ogrinc **Compound specific isotope analysis in food authenticity**

11:45-12:00 T. Grebenc Tractability and quality control in commercial truffles (*Tuber* spp.)

12:00-12:15 L. Strojnik Investigation of truffle aroma compounds: can we differentiate between different species?

12:15-12:30 A. Zitek Towards a multielement and $n(87\text{Sr})/n(86\text{Sr})$ isotope ratio landscape of Austria for food provenancing

12:30-12:45 A. Aguzzioni Geographic traceability of North Italian apples based on Sr isotope ratio and multi-chemical fingerprint

12:45-14:00 Lunch and posters

FOOD AUTHENTICITY AND TRACEABILITY **Moderators: J. Szpunar, N. Poklar Urlih**

14:00-14:30R. Rozenich **Waters DART direct analysis system used for Food Authenticity**

14:30-15:00F. Camin **Traceability and authentication of premium products using stable isotope ratios analysis'**

15:00-15:15 T. Zuliani Elemental content and strontium isotope characterization of Slovenian wine

15:15-15:30 M. Perini $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ analyses of amino acids of grape

15:30-16:00 Coffee break

FOOD QUALITY AND SAFETY **Moderators: E. Heath, F. Camin**

16:00-16:30A. Covaci **"A Bug Lunch" – Evaluating the chemical safety of edible insects**

16:30-16:45 J. Snoj Tratnik Human biomonitoring and its role in food safety: trace elements in men and lactating women living in Slovenia

16:45-17:00 A. Stajnko Diet related exposure to As in Slovenian-Croatian women population (PHIME-CROME study)

17:00-17:15 H. Prosen Determination of neonicotinoid pesticides in Slovenian honey

17:15-17:30 U. Blaznik Ongoing work on implementing cumulative exposure assessment to determine chemical food safety in Slovenia

17:30-17:45 A. Bogožalec Košir Novel molecular approaches for quantification of genetically modified organisms

17:45-18:00 A. Runkel Exposure to endocrine disrupting chemicals via food: Urinary phthalate metabolites in the Slovenian general population

18:00-19:30 Poster session

Tuesday, April 2, 2019

**FOOD QUALITY AND
SAFETY**

Moderators: M. Horvat, A. Lobnik

8:30-9:00	J. Szpunar	Mass spectrometry techniques for the speciation and isotopic composition of selenium in food
9:00-9:30	F. Vanhaecke	Acquiring enhanced insight into the sources of marine Hg pollution, the associated risks and in vivo Hg metabolism via high-precision Hg isotopic analysis with CVG-MC-ICP-MS
9:30-9:45	R. Milačič	Analytical procedures for speciation of zinc, nickel and chromium in food samples
9:45-10:00	K. Vogel-Mikuš	On the toxicity of edible mushrooms – Hg and Se accumulation and bioavailability
10:00-10:15	P. Pongrac	Contrasting allocation of magnesium and calcium in tea leaves may explain their different extraction efficiency
10:15-10:30	M. Černe	Transfer of potentially toxic elements to Chinese cabbage from soil amended with compost derived from the sewage sludge
10:30-11:00	Coffee break	

**FOOD QUALITY AND
SAFETY**

Moderators: D. Heath, S. Novak Krmpotič

11:00-11:30	K. Löschner	Nanoparticles in food – an overview
11:30-12:00	N. Poklar Ulrih	Proliposomal encapsulation of resveratrol
12:00-12:15	A. Lobnik	Novel Optical Chemical Sensors for in situ food quality control
12:15-12:30	V. Golja	Exposure to nano TiO ₂ particles from food contact quasi-ceramic coatings
12:30-12:45	M. Červek	Cattle fattening for top quality beef production
12:45-13:00	H. Elbashir	Interactive effect of gamma radiation and salicylic acid on sprout inhibition of two processing potato varieties
13:00-14:30	Lunch break	
14:30-17:30	Visiting to Sečovelje Salt pens	
15:00-15:30	N. Kovač	A scientific view of the Sečovelje Salina
19:00	Dinner	

Wednesday, April 3, 2019

FOODOMICS

Moderators: A. Schimmelmänn, C. Zoani

9:00-9:30 U. Vrhovšek

Foodomics: a milestone in food and nutritional studies

9:30-9:45 L. Di Donna

Ultra-rapid determination of tyrosol and hydroxytyrosol derivatives in extra virgin olive oil by paper spray tandem mass spectrometry

9:45-10:00 Z. Jandrič

Combined metabolic, elemental and isotopic fingerprinting for origin determination of carrots

10:00-10:30 Coffee break

METROLOGY IN FOOD

Moderators: S. Lojen, U. Vrhovšek

10:30-11:00 A. Schimmelmänn

Development of honey, vegetable oil, flour, and collagen stable isotope reference materials

11:00-11:30 C. Zoani

The role of research infrastructures in supporting Metrology in Food – the experience of METROFOOD-RI

11:30-11:45 R. Jačimović

Calibration and Measurement Capability (CMCs) in food analysis

11:45-12:00 M. Milavec

Key comparison studies on the ratio of the number of copies of specified intact sequence fragments extracted from different matrices

12:00-12:15 E. Bešter

Quantification of measurement uncertainty using model equation: case of determination of alpha-tocopherol content in plant oils

FOOD DATABASES AND SEMANTICS

Moderators: S. Lojen, U. Vrhovšek

12:15-12:45 T. Eftimov

Food and nutrition data management and knowledge discovery

12:45-13:00 U. Pivk Kupirovič

Exploitation of crowdsourcing for collecting compositional data on prepacked foods and guiding healthy food choices with a smart-phone application

13:00-13:15 M. Kos Skubic

Reaction of consumers in Slovenia to the use and understanding of quality labels on food products

13:15-13:45 G. A. Herrmann

A non-analytical solution to supply chain integrity countering fraud

13:45-14:00 Closing ceremony

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Ester Heath

Barbara Korousic Seljak
Maja Remškar
Saša Novak

PROGRAMME

Invited Lectures

	Page
<i>Monday Early Morning Invited Lecture, Monday, Apr 01 2019, 9:15-9:45</i>	
<i>Location: Europa B (12th fl.)</i>	
Session: Invited Lectures	27
Chairs: Nives Ogrinc and Frank Vanhaecke	
9:15 IN-01	A new rapid method for stable isotope analysis of non-exchangeable hydrogen to detect undeclared addition of sugar and sugar syrups to food <i>Simon Kelly, Aiman Abraham, Andrew Cannavan</i>
	27
 <i>Monday Late Morning Invited Lecture, Monday, Apr 01 2019, 11:15-11:45</i>	
<i>Location: Europa B (12th fl.)</i>	
Session: Invited Lectures	27
Chairs: Simon Kelly and Katrin Loeschner	
11:15 IN-02	Compound specific isotope analysis in food authenticity <i>Nives Ogrinc, Andrija R. Ćirić, Doris Potočnik, Lidija Strojnik</i>
	27
 <i>Monday After-lunch Invited Lectures, Monday, Apr 01 2019, 14:00-15:00</i>	
<i>Location: Europa B (12th fl.)</i>	
Session: Invited Lectures	27
Chairs: Joanna Szpunar and Natasa Poklar Ulrih	
14:00 IN-03	Real time food authenticity testing with minimal need for sample preparation using the DART QDa system with LiveID software <i>Rainer Rozenich</i>
	27
14:30 IN-04	Traceability and authentication of premium products using stable isotope ratios analysis <i>Federica Camin, Matteo Perini, Luana Bontempo</i>
	27
 <i>Monday Afternoon Invited Lecture, Monday, Apr 01 2019, 16:00-16:30</i>	
<i>Location: Europa B (12th fl.)</i>	
Session: Invited Lectures	28
Chairs: Ester Heath and Federica Camin	
16:00 IN-05	"A Bug Lunch" - Evaluating the chemical safety of edible insects <i>Giulia Poma, Matthias Cuykx, Elvio Amato, Chiara Calaprice, Francois Focant, Adrian Covaci</i>
	28
 <i>Tuesday Early Morning Invited Lectures, Tuesday, Apr 02 2019, 8:30-9:30</i>	
<i>Location: Europa B (12th fl.)</i>	
Session: Invited Lectures	28
Chairs: Milena Horvat and Aleksandra Lobnik	

8:30 IN-06	Mass spectrometry techniques for the speciation and isotopic composition of selenium in food <i>Joanna Szpunar</i>	28
9:00 IN-07	Acquiring enhanced insight into the sources of marine Hg pollution, the associated risks and in vivo Hg metabolism via high-precision Hg isotopic analysis with CVG-MC-ICP-MS <i>Frank Vanhaecke, Eduardo Bolea-Fernandez, Ana Rua-Ibarz, Sylvia Frantzen, Amund Maage, Monica Sanden, Eva Krupp, Jörg Feldmann</i>	29

Tuesday Late Morning Invited Lectures, Tuesday, Apr 02 2019, 11:00-12:00

Location: Europa B (12th fl.)

Session: **Invited Lectures**

29

Chairs: David Heath and Saša Novak

11:00 IN-08	Nanoparticles in food – an overview <i>Katrin Loeschner</i>	29
11:30 IN-09	Proliposomal encapsulation of resveratrol <i>Natasa Poklar Ulrih</i>	30

Tuesday Invited Lecture at Sečovlje Salt Pens, Tuesday, Apr 02 2019, 15:00-15:30

Location: Sečovlje

Session: **Invited Lectures**

30

15:00 IN-10	A scientific view of the Sečovlje Salina <i>Nives Kovač</i>	30
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Wednesday Early Morning Invited Lecture, Wednesday, Apr 03 2019, 9:00-9:30

Location: Europa B (12th fl.)

Session: **Invited Lectures**

30

Chairs: Arndt Schimmelmann and Claudia Zoani

9:00 IN-11	Foodomics: a milestone in food and nutritional studies <i>Urška Vrhovsek</i>	31
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Wednesday Late Morning Invited Lectures, Wednesday, Apr 03 2019, 10:30-11:30

Location: Europa B (12th fl.)

Session: **Invited Lectures**

31

Chairs: Sonja Lojen and Urška Vrhovsek

10:30 IN-12	Development of honey, vegetable oil, flour, and collagen stable isotope reference materials <i>Arndt Schimmelmann, Federica Camin, Simon Kelly, Nives Ogrinc</i>	31
11:00 IN-13	The role of research infrastructures in supporting Metrology in Food – the experience of METROFOOD-RI <i>Claudia Zoani</i>	31

Wednesday Afternoon Invited Lecture, Wednesday, Apr 03 2019, 12:15-12:45

Location: Europa B (12th fl.)

Session: **Invited Lectures**

32

Chairs: Sonja Lojen and Urska Vrhovsek

12:15 **Food and nutrition data management and knowledge discovery**

32

IN-14 *Tome Eftimov*

Wednesday Closing Invited Lecture, Wednesday, Apr 03 2019, 13:15-13:45

Location: Europa B (12th fl.)

Session: **Invited Lectures**

32

Chairs: Sonja Lojen and Urska Vrhovsek

13:15 **A non-analytical solution to supply chain integrity countering fraud**

32

IN-15 *Gerald A. Herrmann*

Oral Presentations

Page

Monday Early Morning Session, Monday, Apr 01 2019, 9:45-10:45

Location: Europa B (12th fl.)

Session: **Food Authenticity and Traceability**

37

Chairs: Nives Ogrinc and Frank Vanhaecke

9:45	State of the art Isotope Ratio Mass Spectrometry (IRMS) applied to food traceability	37
OR-01	<i>Lionnel Mounier, Dave Wanless</i>	
10:00	Authentication of the geographical origin of hops (<i>Humulus lupulus</i> L.)	37
OR-02	<i>Miha Ocvirk, Iztok J. Košir, Marijan Nečemer, Nives Ogrinc</i>	
10:15	Comparison of organic and conventional beer in view of the content of the selected	37
OR-03	phytoestrogens <i>Jelena B. Golubović, Ester Heath, Iztok J. Košir, David J. Heath</i>	
10:30	Isotopic and multi-elemental signatures as indicators of origin of sparkling wines	38
OR-04	<i>Robin Cellier, Sylvain Bérail, Julien Barre, Ekaterina N. Epova, Olivier F.X. Donard</i>	

Monday Late Morning Session, Monday, Apr 01 2019, 11:45-12:45

Location: Europa B (12th fl.)

Session: **Food Authenticity and Traceability**

38

Chairs: Simon Kelly and Katrin Loeschner

11:45	Tractability and quality control in commercial truffles (<i>Tuber</i> spp.) – a need or an	38
OR-05	unachievable aim? <i>Tine Grebenc, Lidija Strojnik, Bor Krajnc, Tina Unuk, Nives Ogrinc</i>	
12:00	Investigation of truffle aroma compounds: can we differentiate between different	38
OR-06	species? <i>Lidija Strojnik, Tine Grebenc, Nives Ogrinc</i>	
12:15	Towards a multielement and n(87Sr)/n(86Sr) isotope ratio landscape of Austria for	39
OR-07	food provenancing <i>Andreas Zitek, Melanie Diesner, Anastassiya Tchaikovsky, Oppen Christine, Stephan Hann, Thomas Prohaska</i>	
12:30	Geographic traceability of North Italian apples based on Sr isotope ratio and multi-	39
OR-08	chemical fingerprint <i>Agnese Aguzzoni, Michele Bassi, Emanuela Pignotti, Peter Robatscher, Francesca Scandellari, Werner Tirler, Massimo Tagliavini</i>	

Monday After-lunch Session, Monday, Apr 01 2019, 15:00-15:30

Location: Europa B (12th fl.)

Session: **Food Authenticity and Traceability**

40

Chairs: Joanna Szpunar and Natasa Poklar Ulrih

15:00	Elemental content and strontium isotope characterisation of Slovenian wine	40
OR-09	<i>Tea Zuliani, Martina Furdek Turk, Ana Drinčić, Ekaterina N. Epova, Sylvain Bérail, Olivier F.X. Donard</i>	

15:15	$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ analyses of amino acids of grape	40
OR-10	<i>Matteo Perini, Lidiya Strojnik, Federica Camin</i>	

Monday Afternoon Session, Monday, Apr 01 2019, 16:30-18:00

Location: Europa B (12th fl.)

Session: **Food Quality and Safety**

40

Chairs: Ester Heath and Federica Camin

16:30	Human biomonitoring and its role in food safety: trace elements in men and lactating women living in Slovenia	41
OR-11	<i>Janja Snoj Tratnik, Ingrid Falnoga, Darja Mazej, David Kocman, Vesna Fajon, Marta Jagodic, Anja Stajniko, Ajda Trdin, Zdenka Šlejkovec, Zvonka Jeran, Joško Osredkar, Alenka Sešek-Briški, Mladen Krsnik, Alfred B. Kobal, Lijana Kononenko, Milena Horvat</i>	
16:45	Diet related exposure to As in Slovenian-Croatian women population (PHIME-CROME study)	41
OR-12	<i>Anja Stajniko, Zdenka Šlejkovec, Darja Mazej, Janja Snoj Tratnik, Alenka France Štiglic, Alenka Sešek-Briški, Igor Prpić, Zdravko Špirić, Janja Marc, Milena Horvat, Ingrid Falnoga</i>	
17:00	Determination of neonicotinoid pesticides in Slovenian honey	41
OR-13	<i>Miha Mrzlikar, David J. Heath, Ester Heath, Jernej Markelj, Helena Prosen</i>	
17:15	Ongoing work on implementing cumulative exposure assessment to determine chemical food safety in Slovenia	42
OR-14	<i>Urška Blaznik</i>	
17:30	Novel molecular approaches for quantification of genetically modified organisms	42
OR-15	<i>Alexandra Bogožalec Košir</i>	
17:45	Exposure to endocrine disrupting chemicals via food: Urinary phthalate metabolites in the Slovenian general population	43
OR-16	<i>Agneta Runkel, Darja Mazej, Janja Snoj Tratnik, Milena Horvat</i>	

Tuesday Early Morning Lectures, Tuesday, Apr 02 2019, 9:30-10:30

Location: Europa B (12th fl.)

Session: **Food Quality and Safety**

43

Chairs: Milena Horvat and Aleksandra Lobnik

9:30	Analytical procedures for speciation of zinc, nickel and chromium in food samples	43
OR-17	<i>Radmila Milačič, Tea Zuliani, Kelly Peeters, Breda Novotnik, Dušan Žigon, Janez Ščančar</i>	
9:45	On the toxicity of edible mushrooms – Hg and Se accumulation and bioavailability	44
OR-18	<i>Katarina Vogel-Mikuš, Anja Kavčič, Johannes Teun van Elteren, Marta Debeljak, Iztok Arčon, Alojz Kodre</i>	
10:00	Contrasting allocation of magnesium and calcium in tea leaves may explain their different extraction efficiency into the infusion	44
OR-19	<i>Paula Pongrac, Mitja Kelemen, Primož Vavpetič, Primož Pelicon, Roser Tolrà, Roghieh Hajiboland, Juan Barceló, Charlotte Poschenrieder, Marjana Regvar, Katarina Vogel-Mikuš</i>	
10:15	Transfer of potentially toxic elements to Chinese cabbage from soil amended with compost derived from the sewage sludge	45
OR-20	<i>Marko Černe, Igor Palčič, Igor Pasković, Nikola Major, Zoran Užila, Josipa Perković, Smiljana Goreta Ban, Vilim Filipović, Marija Romić, Radojko Jaćimović, Ljudmila Benedik, Dean Ban</i>	

Tuesday Late Morning Lectures, Tuesday, Apr 02 2019, 12:00-13:00

Location: Europa B (12th fl.)

Session: **Food Quality and Safety**

45

Chairs: David Heath and Saša Novak

12:00	Optical chemical sensors for food quality	45
OR-21	<i>Aleksandra Lobnik, Polonca Nedeljko, Tinkara Mastnak</i>	
12:15	Exposure to nano TiO₂ particles from food contact quasi-ceramic coatings	46
OR-22	<i>Viviana Golja, Goran Dražić, Saša Novak</i>	
12:30	Cattle fattening for top quality beef production	46
OR-23	<i>Matjaž Červek</i>	
12:45	Interactive effect of gamma radiation and salicylic acid on sprout inhibition of two	46
OR-24	processing potato varieties <i>Hind Elbashir</i>	

Wednesday Early Morning Lectures, Wednesday, Apr 03 2019, 9:30-10:00

Location: Europa B (12th fl.)

Session: **Foodomics**

46

Chairs: Arndt Schimmelmann and Sonja Lojen

9:30	Ultra-rapid determination of tyrosol and hydroxytyrosol derivatives in extra virgin	46
OR-25	olive oil by paper spray tandem mass spectrometry <i>Lucia Bartella, Fabio Mazzotti, Iole Gabriele, Giovanni Sindona, Leonardo Di Donna</i>	
9:45	Combined metabolic, elemental and isotopic fingerprinting for origin determination	47
OR-26	of carrots <i>Zora Jandric, Andreas Zitek, Tim Causon, Václav Štursa, Anastasiya Tchaikovsky, Thomas Prohaska, Stephan Hann</i>	

Wednesday Late Morning Lectures, Wednesday, Apr 03 2019, 11:30-12:15

Location: Europa B (12th fl.)

Session: **Metrology in Food**

47

11:30	Calibration and measurement capabilities (CMCs) for food sample analysis	47
OR-27	<i>Radojko Jaćimović, Tea Zuliani, Vesna Fajon, Polona Vreča, Milena Horvat</i>	
11:45	Key comparison studies on the ratio of the number of copies of specified intact se-	48
OR-28	quence fragments extracted from different matrices <i>Mojca Milavec</i>	
12:00	Quantification of measurement uncertainty using model equation: Case of determi-	48
OR-29	nation of alpha-tocopherol content in plant oils <i>Erika Bešter, Bojan Butinar, Milena Bučar-Miklavčič</i>	

Wednesday Afternoon Lectures, Wednesday, Apr 03 2019, 12:45-13:15

Location: Europa B (12th fl.)

Session: **Food Databases and Semantics**

49

12:45 OR-30	Exploitation of crowdsourcing for collecting compositional data on prepacked foods and guiding healthy food choices with a smart-phone application <i>Urška Pivk Kupirovič, Anita Kušar, Barbara Koroušić Seljak, Igor Pravst</i>	49
13:00 OR-31	Slovenian consumers' preferences towards traditional food products with national and EU quality labels <i>Mira Kos Skubic, Marija Klopčič, Mira Kos Skubic, Anita Ule, Karmen Erjavec</i>	49

Poster Presentations

		Page
<i>Lunch Break and Poster Session, Monday, Apr 01 2019, 12:45-14:00</i>		
<i>Location: Europa D (12th fl.)</i>		
Session: Posters		53
PO-01 B-01	Geographical origin of plant food: Implementation of robust screening tests <i>Anja Mahne Opatić, Marijan Nečemer, Sonja Lojen, Rajko Vidrih</i>	53
PO-02 B-02	On the traces of the <i>Cannabis sativa</i> <i>Marco Calvi, Barbara Stenni, Federica Camin, Luana Bontempo, Mario Baldini, Nives Ogrinc, Tea Zuliani, Paolo Guarnaccia, Clara Turetta</i>	53
PO-03 B-03	Application of chemometric tools for determination of authenticity of Serbian pear spirits <i>Biljana Marosanovic, Maja Lojovic, Aleksandra Bauer</i>	54
PO-04 B-04	Characterization of truffles (<i>Tuber</i> sp.) in Slovenia using stable isotope approach and elemental composition <i>Bor Krajnc, Marijan Nečemer, Federica Camin, Katarina Vogel-Mikuš, Staša Hamzić Gregorčič, Lidija Strojnik, Nives Ogrinc</i>	54
PO-05 B-05	Establishing reproducibility in compound-specific stable isotope measurements for food authentication and adulteration <i>Chris Yarnes</i>	54
PO-06 B-06	Development of HILIC method for LC-MS analysis of glycolysis metabolites <i>Anže Pavlin, Martin Šala, Drago Kočar</i>	55
PO-07 B-07	Trust me? Consumer trust in expert information on food product labels <i>Christoph Rupprecht, Lei Fujiyoshi, Steven McGreevy, Ichiro Tayasu</i>	55
PO-08 B-08	Influence of the isotope signature of new fertilizers in the nitrogen isotope fingerprint of the organic foodstuffs <i>Jose Manuel Moreno-Rojas, Francisco Cuevas, Jose Carlos Montenegro</i>	56
PO-09 B-09	Characterisation of white truffle using ICP-OES and IRMS <i>Luana Bontempo, Federica Camin, Luca Ziller, Roberto Larcher</i>	56
PO-10 B-10	Food and beverage fraud prevention using isotope fingerprints <i>Mario Tuthorn, Christopher Brodie, Dieter Juchelka, Oliver Kracht, Jens Radke</i>	57
PO-11 B-11	Neutron Activation Analysis for the characterization and discrimination of processed animal proteins and insect meal <i>Luigi Bergamaschi</i>	57
PO-12 B-12	An isotope analysis of the link between groundwater and milk in the Parmigiano-Reggiano cheese production area for food traceability <i>Marta Boito, Paola Iacumin, Nives Ogrinc</i>	57
PO-13 B-13	Phenolic profile of two autochthonous grape varieties from Serbia <i>Milica Sredojević, Dragana Dabić Zagorac, Uroš Gašić, Ivanka Ćirić, Maja Natić</i>	58
PO-14 B-14	Assessment of differences among conventional and organic production of fruits based on phenolic profiles <i>Maja Natić, Dragana Dabić Zagorac, Jasminka Milivojević, Mekjell Meland, Milica Fotirić Akšić</i>	58

PO-15 B-15	Improved detection of exogenous sugars addition to fruit juices by rapid determination of sugars $\delta^{13}\text{C}$ with HPLC-co-IRMS <i>Ni-Na Chang, Jung-Tai Lu, Nai-Jung Wan</i>	58
PO-16 B-16	Optimization of on-line bulk and compounds specific carbon isotope ratio measurements of foods and beverages by using flow injection/liquid chromatography isotope ratio mass spectrometry (FI/LC-IRMS) <i>Nai-Jung Wan</i>	59
PO-17 B-17	Determination of geographical origin of different fruit and vegetables using stable isotope ratio mass spectrometry and element analysis <i>Andrija R. Ćirić, Lidija Strojnik, Marijan Nečemer, Bernard Ženko, Nives Ogrinc</i>	59
PO-18 B-18	Characterization of Spirulina food supplements from Slovenian market through element composition and stable isotope ratio of light elements <i>Jasmina Masten, Marta Jagodic, Lidija Strojnik, Marijan Nečemer, Katarina Vogel-Mikuš, Nives Ogrinc</i>	60
PO-19 B-19	Characterisation of bottled waters by stable isotope and multi-elemental analysis <i>Tea Zuliani, Tjaša Kanduč, Polona Vreča</i>	60
PO-20 B-20	Nuclear magnetic resonance in food safety and quality <i>Monika Škrjanc, Damjan Makuc, Janez Plavec</i>	61
PO-21 B-21	Potential of stable carbon isotope analysis for compliance checking of corn mixed bread <i>Mojca Korošec, Laura Slemc, Nives Ogrinc</i>	61
PO-22 B-22	How to establish stable isotope databases for authenticity assessment of aromas <i>Lidija Strojnik, Jože Hladnik, Nika Weber, Darinka Koron, Matej Stopar, Emil Zlatič, Doris Kokalj, Mateja Naglič Gril, Nives Ogrinc</i>	62
PO-23 B-23	Using stable isotope databases for authenticity assessment of commercial flavoured products <i>Lidija Strojnik, Matteo Perini, Silvia Pianezze, Federica Camin, Nives Ogrinc</i>	62
PO-24 B-24	Parameters for discrimination between organic and conventional production: a case study for chicory (<i>Cichorium intybus</i> L.) <i>Lovro Sinkovič, Marijan Nečemer, Nives Ogrinc, Vladimir Meglič</i>	62
PO-25 B-25	Evaluation of carbon and nitrogen isotope analysis for authentication of organic versus conventional pork products <i>Monica Rosell, Roget Puig, Raul Carrey, Carme Audi-Miro, Sara Herrero-Martin, Joan Parareda-Sala, Valeri Salgot-Nadal, Albert Soler</i>	63
PO-26 B-26	Can isotopic fingerprinting distinguish unequivocally among Spanish beers? <i>Monica Rosell, Raul Carrey, Sara Herrero-Martin, Albert Soler</i>	63
PO-27 B-27	Arsenic compounds in rice and rice drinks available on the Slovene market <i>Zdenka Šlejkovec, Leon Gorše, Ana Grobler, Zala Resnik Poljašević, Rok Kvartuh, Marinka Kovač, Mirna Malus, Marta Jagodic, Ingrid Falnoga</i>	64
PO-28 B-28	Exposure to mercury during prenatal period <i>Ajda Trdin, Janja Snoj Tratnik, Igor Prpić, Zdravko Špirić, Ingrid Falnoga, Milena Horvat</i>	64
PO-29 B-29	Optimization ultrasound-assisted method for extraction bioactive compounds from garlic (<i>Allium sativum</i>) sample using response surface methodology <i>Andrija R. Ćirić, Lidija Strojnik, Nives Ogrinc</i>	65

PO-30 B-30	Comparison of new developed non-esterification with standard method for determination fatty acids in milk sample <i>Doris Potočnik, Andrija R. Ćirić, Nives Ogrinc</i>	65
PO-31 B-31	Migration of bisphenol and their derivatives from food contact material by target and untargeted analyses <i>Ana Kovačič, Ester Heath, Celine Gys, Tina Kosjek, Adrian Covaci, David J. Heath</i>	66
PO-32 B-32	Bisphenols removal in algal wastewater treatment bioreactors – future prospects of product reuse <i>David Škufca, Franja Prosenc, Ana Kovačič, Marilyn Pflieger, Tina Kosjek, Tjaša Griessler Bulc, Ester Heath</i>	66
PO-33 B-33	Effects of soil properties, management practices and rice genotypes on Arsenic and Cadmium accumulation in rice <i>Gian M. Beone, Marco Romani, Umberto Rolla, Maria Martin, Terenzio Bertuzzi, Maria C. Fontanella</i>	67
PO-34 B-34	Stability of phenolic compounds during simulated in vitro gastrointestinal digestion of wild raspberries <i>Nevena R. Mihailović, Vladimir B. Mihailović, Nikola Z. Srečković, Ljubinka G. Joksović</i>	67
PO-35 B-35	Comparison of algae dietary supplements: antioxidative potencial and origin <i>Jan Kejžar, Marta Jagodic, Marijan Nečemer</i>	68
PO-36 B-36	Uranium radioisotopes and Po-210 content in soil and cabbage (<i>Brassica oleracea</i>) samples collected in the vicinity of a former uranium mine <i>Miha Trdin, Špela Kavčič, Janez Sušin, Vida Žnidaršič Pongrac, Janez Bergant, Ljudmila Benedik</i>	68
PO-37 B-37	Proposal for a european metrology network on food safety - EMN-FS <i>Francesca Durbiano, Andrea Mario Rossi</i>	69
PO-38 B-38	Moniliformin determination by ligand chromatography using lanthanide ions <i>Terenzio Bertuzzi, Silvia Rastelli, Annalisa Mulazzi</i>	69
PO-39 B-39	Targeted healthy and undesirable compounds in small and large scale brewed beers <i>Terenzio Bertuzzi, Annalisa Mulazzi, Filippo Rossi, Giorgia Spigno</i>	69
PO-40 B-40	Effect of simulated gastrointestinal digestion on phenolic compounds in <i>Lythrum salicaria</i> L. extracts <i>Vladimir B. Mihailović, Nikola Z. Srečković, Jelena Katanić</i>	70
PO-41 B-41	Liquid chromatographic-tandem mass spectrometric method for determination of tetrodotoxin in mussels <i>Zlatka Bajc, Renata Ciglarich, Andrejka Močnik, Jožica Dolenc</i>	70
PO-42 B-42	Validation of a confirmatory multi-residue method for the determination of thirteen hormones in several bovines matrices using HPLC-MS/MS <i>Fadl Moussa, Samia Mokh, Nicola Bernabò, Mohamad Al Iskandarani</i>	71
PO-43 B-43	A new method for determination of monomethylmercury based on separation of Hg dithizonates and cold vapour atomic absorption detection <i>Vesna Fajon, Hirokatsu Akagi, Milena Horvat</i>	71
PO-44 B-44	Analysis of titanium dioxide nanoparticles in food by ICP-MS/MS in single particle mode <i>Janja Vidmar, Sara Lopéz-Sanz, Laurent Devoille, Johanna Noireaux, Manuel Correia, Katrin Loeschner</i>	72

PO-45 B-45	Biodegradable food packaging materials containing substances of plant origin <i>Malgorzata Latos-Brozio, Anna Masek</i>	72
PO-46 B-46	“Green” nanotechnologies: Synthesis of silver nanoparticles using <i>Lythrum salicaria</i> L. extracts and study of their biological activity <i>Nikola Z. Srecković, Zoran Nedić, Jelena Katanić, Vladimir B. Mihailović</i>	72
PO-47 B-47	Antimicrobial activity of molybdenum oxide nanoparticles and their potential for application in food industry <i>Urška Gradišar Centa, Meta Sterniša, Sonja Smole Možina, Maja Remškar</i>	73
PO-48 B-48	Development of an efficient passive sampler adsorbent for the detection of mercury in water via stratified nanostructured knitting <i>Raghuraj S. Chouhan, Vesna Fajon, Igor Živković, Majda Pavlin, Sabina Berisha, Ivan Jerman, David J. Heath, Milena Horvat</i>	73
PO-49 B-49	Are there titania nanoparticles in my food? <i>Saša Novak, Viviana Golja, Anja Drame</i>	74
PO-50 B-50	Fast analysis of alpha-tocopherol in olive oil by Paper Spray tandem mass spectrometry and isotope dilution method <i>Fabio Mazzotti, Lucia Bartella, Leonardo Di Donna, Giovanni Sindona</i>	74
PO-51 B-51	Effect of yeast cells exposure to fermented <i>Spirulina</i> on their protein profile: Characterization of yeast lysates using high resolution Orbitrap mass spectrometry <i>Jasmina Masten, Berta Cillero-Pastor, Ronny Mohren, Nives Ogrinc, Polona Jamnik</i>	74
PO-52 B-52	Toxic elements in whey protein supplements <i>Silvia Mallia, Sharona Perrin</i>	75
PO-53 B-53	Dietary habits of Slovenian mothers and breast milk fatty acid composition: comparison of inland and coastal areas <i>Marta Jagodic, Janja Snoj Tratnik, Doris Potočnik, Darja Mazej, Nives Ogrinc, Milena Horvat</i>	75
PO-54 B-54	Missing data imputation methods for food composition data <i>Gordana Ispirova, Tome Eftimov, Barbara Koroušić Seljak</i>	76
PO-55 B-55	Proficiency test PT mushroom - trace elements in mushroom <i>Vesna Fajon, Milena Horvat, Igor Živković, Radojko Jaćimović, Jože Kotnik</i>	76
PO-56 B-56	The isotopic approach to the authenticity of Cypriot traditional products <i>Elena Ioannou Papayianni, Rebekka Kokkinofa</i>	77
PO-57 B-57	The application of isotopic and other techniques to verify the declaration of apple juices on Slovenian market <i>Karmen Bizjak Bat, Klemen Eler, Branka Mozetič Vodopivec, Ines Mulič, Nives Ogrinc</i>	77
PO-58 B-58	Comparison of cereal species according to the content of free asparagine and sugars as reactants in the Maillard reaction <i>Sladjana Žilić, Primož Titan, Dejan Dodig, Zorica Basić</i>	77
PO-59 B-59	Colored maize and wheat as widely grown natural sources of anthocyanins <i>Sladjana Žilić, Primož Titan, Dejan Dodig, Jelena Vančetović, Nikola Grčić</i>	78

INVITED
LECTURES'
ABSTRACTS

Invited Lectures

Monday Early Morning Invited Lecture

Apr 01, 9:15 - 9:45

IN-01

A new rapid method for stable isotope analysis of non-exchangeable hydrogen to detect undeclared addition of sugar and sugar syrups to food

Simon Kelly, Aiman Abraham, Andrew Cannavan

IAEA Food and Environmental Protection Laboratory (FEPL), Austria

An improved procedure for the isotope analysis of the non-exchangeable hydrogen in mono and disaccharides has been developed to demonstrate the feasibility of detecting undeclared addition of C3 and C4 sugar products in foods and beverages susceptible to adulteration. The procedure utilizes a simple one-step reaction, with the derivatising agent N-methyl-bis-trifluoroacetamide, to substitute the exchangeable hydroxyl-hydrogen with trifluoroacetate derivatives that are sufficiently volatile to be separated and measured by gas chromatography coupled to isotope ratio mass spectrometry. The conversion of the derivatised sugars into the measuring gas is achieved using a high temperature chromium reactor that retains carbon, oxygen and fluorine whilst releasing hydrogen gas for stable isotope measurement. The new procedure has advantages over methods using nitro-sugar derivatives and degradation products, such as hexamethylenetetramine and calcium formate, in terms of ease of use, analysis time and sensitivity. The differences between the $\delta^2\text{H}$ values of the non-exchangeable hydrogen in sugars from fruit juices and honey and those from beet and cane sugars/syrups permits the presence of these potential adulterants to be rapidly detected.

Invited Lectures

Monday Late Morning Invited Lecture

Apr 01, 11:15 - 11:45

IN-02

Compound specific isotope analysis in food authenticity

Nives Ogrinc, Andrija R. Ćirić, Doris Potočnik, Lidija Strojnik

Jožef Stefan Institute, Department of Environmental Sciences, Slovenia

The aim of this presentation is to provide an overview of compound-specific isotope analysis (CSIA) in food authenticity and traceability. First the theoretical background will be presented with most common analytical approaches including GC-C-IRMS and LC-IRMS followed by the review of analytical challenges associated with chromatographic separation and derivatization. Selected applications and examples will include the use of CSIA in

traceability studies of olive oil, authenticity of aroma compounds and honey and discrimination between organically and conventionally produced products.

It was found that the discrimination between olive oil according to the country of origin was better when stable isotope composition of carbon in fatty acids were included in statistical evaluation of the data. The application of CSIA in authenticity of aroma compound originating from fruits and vanilla enable us to distinguish natural versus synthetic origin. Indeed, cheap, synthetic, petroleum-derived compounds usually have different isotope values than their more costly, natural counterparts. This approach is more efficient if both carbon and hydrogen stable isotopes are used. Coupling high performance liquid chromatography (HPLC) with isotope ratio mass spectrometry (LC-IRMS) has the unrivaled advantage of the simultaneous determination of $\delta^{13}\text{C}$ values from glucose, fructose, di-, tri-, and oligo-saccharides, allowing the detection of more sophisticated honey adulteration. The last part of the presentation will show how CSIA of amino acids could be used to discriminate between conventionally and organically grown food commodities such as wheat.

Invited Lectures

Monday After-lunch Invited Lectures

Apr 01, 14:00 - 15:00

IN-03

Real time food authenticity testing with minimal need for sample preparation using the DART QDa system with LiveID software

Rainer Rozenich

Waters GmbH, Austria

DART (Direct Analysis in Real Time) is atmospheric pressure chemical ionization for direct analysis without the need for chromatographic separation. It enables a rapid, sample preparation-free solution for informed and immediate sample classification.

It is applicable for point of control quantitative testing, with reduced sample manipulation giving accurate mass spectral information in a second. There are many potential applications for the DART QDa System with LiveID within food safety, quality and research, including quality testing of raw ingredients, in-process products, and finished product quality control and authenticity.

IN-04

Traceability and authentication of premium products using stable isotope ratios analysis

Federica Camin, Matteo Perini, Luana Bontempo

Fondazione Edmund Mach (FEM), Italy

Determining the authenticity of foods means uncovering misdescription of foods not meeting the requirements for adopting a particular name, substitution with cheaper but similar ingredients, undeclared processes and/or extension of food using adulterants, incorrect origin (e.g.

geographical), species or production method (e.g. organic vs conventional). Nowadays, the objective assessment of food authenticity is of paramount importance as consumers come into daily contact with a wide variety of foods. Indeed, globalization means that more and more foods are traded around the world.

Methods for testing authenticity and providing analytical data on traceability require robust analytical techniques that can be used by the various regulatory authorities. Of the many techniques available, one of the most widely-used is isotope ratio mass spectrometry (IRMS), applied since around 1975 to detect adulteration of products like wine, honey, fruit juice, maple syrup, vinegar with cheaper extenders, such as sugar, or simply water. Those “traditional” applications in food control rely on the analysis of the stable isotope ratios of only one or two elements ($^{13}\text{C}/^{12}\text{C}$ and/or $^{18}\text{O}/^{16}\text{O}$, $^{13}\text{C}/^{12}\text{C}$ and $2\text{H}/1\text{H}$) and several of these methods have been officially validated and acknowledged as AOAC, CEN, EU or OIV methods. More recently multi-isotope ratio analysis ($^{13}\text{C}/^{12}\text{C}$, $^{18}\text{O}/^{16}\text{O}$, $2\text{H}/1\text{H}$, $^{15}\text{N}/^{14}\text{N}$, $^{34}\text{S}/^{32}\text{S}$, sometimes combined with elemental or NMR profiling) have been successfully applied for verifying geographical origin of food (e.g. PDO cheese, olive oil, cacao). Other applications concern identification of the natural origin of flavours (e.g. vanillin) and of nutraceutical and pharmaceutical products (e.g. Red Yeast Rice, serenoa oil, squalene).

Invited Lectures

Monday Afternoon Invited Lecture Apr 01, 16:00 - 16:30

IN-05

”A Bug Lunch” - Evaluating the chemical safety of edible insects

Giulia Poma¹, Matthias Cuykx¹, Elvio Amato¹, Chiara Calaprice², Francois Focant², Adrian Covaci¹

¹University of Antwerp, Belgium

²University of Liège, Belgium

Due to the rapid increase in world population, the use of alternative and more environmentally sustainable food sources is strongly encouraged. In this perspective, the opportunity for edible insects to offer a valuable alternative for the rising demand for meat or fish products is remarkable¹. However, like other animal products, insects might accumulate hazardous chemicals². The present pilot study aimed to provide a comprehensive overview of the residual levels of several classes of chemical compounds (including halogenated and phosphorous flame retardants, HFRs, PFRs; PCBs; organochlorine compounds, OCPs; dioxins and furans; metals) in four species of edible insects and four insect-based foodstuffs currently commercialized in Belgium.

Our results pointed out a generally low chemical contamination of the analyzed samples. The levels of PCBs and OCPs ranged from 27 to 2,065 pg/g and from 46 to 368 pg/g, respectively. Total HFRs were below 36 pg/g,

while the total concentrations of PFRs were between 783 and 23,786 pg/g. Total dioxin and furan concentrations ranged from 0.05 to 0.3 pg WHO-TEQ/g, mostly below the regulated maximum residual levels set for beef, poultry, fish, milk and eggs. Cu (0.9-9.1 mg/kg) and Zn (6.4 to 59 mg/kg) were consistently the most abundant metals, suggesting that edible insects have the potential to provide these essential micronutrients.

Finally, the chemical concentrations measured in the analyzed samples were compared with those found in several commonly consumed food groups in Belgium^{3,4} and worldwide. Overall, our results support the possibility for humans to consume these insect species with no additional hazards in comparison with commonly eaten animal products and indicate that the analyzed insect food could be considered a valuable alternative to common sources of proteins.

[1] FAO. Edible insects. Future prospects for food and feed security; 2013; Vol. 171.

[2] Poma, G. et al. Food Chem. Toxicol. 2017, 100, 70–79.

[3] Poma, G. et al. Chemosphere 2018, 194, 256–265.

[4] Poma, G. et al. Environ. Sci. Technol. 2018, 52 (4), 2331–2338.

Invited Lectures

Tuesday Early Morning Invited Lectures Apr 02, 8:30 - 9:30

IN-06

Mass spectrometry techniques for the speciation and isotopic composition of selenium in food

Joanna Szpunar

Centre for Scientific Research CNRS IPREM, France

Selenium is an element with a narrow concentration gap between the toxicity and the essentiality, often evoked in the context of antioxidant protection and cancer prevention [1]. Quantitative information on the concentrations of individual Se forms in natural and Se-enriched (functional) foodstuffs is of paramount importance for the evaluation of its bioavailability, and thus the quality and safety of commercial products. A particular importance has been attributed to the Se metabolic pathways leading to the synthesis of physiologically important selenoproteins and low (< 1000 Da) molecular weight selenometabolites. It can be the basis of the determination of the provenance of Se-enriched supplements (especially yeast) along with the Se isotopic composition.

The discrimination among the different Se chemical forms present in biological systems is a challenge for analytical chemists because - besides having several possible oxidation states in its inorganic form - selenium can be incorporated into tens of known (and probably many more not yet discovered) selenometabolites, many SeCys- and SeMet-containing proteins, selenosugars, selenolipids

and, the last but not least, it can be present as Se nanoparticles. The study of the isotopic composition is not trivial either because of the numerous polyatomic interferences in an argon plasma (e.g. ICP MS).

Developments in Se speciation analysis by coupling separation techniques with parallel elemental and molecular mass spectrometry have resulted in the identification and quantification of a growing number of Se species opening a way to comprehensive species-selective characterization of biological samples, including foodstuffs of plant and animal origin. The recently demonstrated presence of nanoparticulate selenium raises questions about its determination.

The lecture discusses, in particular, the progress in ESI FT MS. The technique offers high resolution and mass accuracy and large intrascan dynamic range and has made structure elucidation of Se-species a quasi-routine task. At the same time, the high-throughput data processing, and especially automatic extraction of element-specific data from HPLC-ESI MS data sets, allows ESI MS to be considered as an element specific detector. Electrospray MS starts outpacing ICP MS in terms of detection limits with the advantage of the possibility to use the multiple reaction mode for the species quantification even in the case of incomplete separations. In terms of isotopic composition the use of hydride generation ICP multicollector MS and the potential of alternative plasma sources and ESI with Orbitrap MS will be highlighted. Advances in the analysis for nanoparticulate selenium by single-particle ICP MS will be briefly summarized.

[1] D.L. Hatfield, U. Schweizer, P.A. Tsuji, V.N. Gladyshev, Selenium. Its Molecular Biology and Role in Human Health, Springer, 2016., Springer, 2016.

IN-07

Acquiring enhanced insight into the sources of marine Hg pollution, the associated risks and in vivo Hg metabolism via high-precision Hg isotopic analysis with CVG-MC-ICP-MS

Frank Vanhaecke¹, Eduardo Bolea-Fernandez¹, Ana Ruabaraz¹, Sylvia Frantzen², Amund Maage³, Monica Sanden², Eva Krupp⁴, Jörg Feldmann⁴

¹Ghent University, Department of Chemistry, Belgium

²Institute of Marine Research, Norway

³University of Bergen, Faculty of Mathematics and Natural Sciences, Norway

⁴University of Aberdeen, Department of Chemistry, United Kingdom

Mercury (Hg) is one of the most important global pollutants. It occurs in the environment as a result of natural and anthropogenic emission and both wildlife and humans are exposed to the harmful effects of this highly toxic heavy metal and its compounds. Anthropogenic Hg emissions resulting from a variety of processes, such as coal combustion, metal refining and manufacturing, cement production and waste incineration, are the main contributors to the world-wide increasing Hg levels. Hg is globally distributed through the atmosphere, atmospheric deposition being the largest source of Hg to the oceans.

In aquatic ecosystems, inorganic Hg can be converted into methylmercury (MeHg), the most toxic Hg species. MeHg can be bioaccumulated and biomagnified across food chains, making seafood consumption the prime source of human Hg intake. The maximum allowable level of Hg in seafood is 0.5 mg kg⁻¹ wet weight.

A team of researchers from Ghent University (Belgium) and the Institute of Marine Research (Norway) have used a combination of quantitative Hg determination using single-collector ICP-mass spectrometry (ICP-MS), Hg speciation (MeHg determination) using gas chromatography - ICP-MS (GC-ICP-MS) and high-precision Hg isotopic analysis using cold vapor generation - multicollector ICP-MS (CVG-MC-ICP-MS) for characterizing tissues from marine species in specific contexts.

To assess the introduction of metallic Hg leaking from a WWII submarine wreck (U-864) located close to the Norwegian coast into the marine food web, white meat (muscle tissue) and brown meat (predominantly consisting of the digestive system and the gonads) of brown crab (*Cancer pagurus*) was studied. Differences in the speciation and isotopic analysis of the Hg in both tissue types suggested absence of methylation of the metallic Hg under the prevailing conditions.

A similar approach was used for studying Hg pollution along the Norwegian coast, relying on the analysis of liver and muscle tissue of tusk (*Brosme brosme*). Results for tusks caught at the U-864 location confirmed the hypothesis formulated on the basis of the observations made for brown crab (*Cancer pagurus*). Careful evaluation of the data for tusks from a fjord pointed to a Zn smelter as the source of local Hg pollution.

A team of researchers from Ghent University (Belgium) and the University of Aberdeen (UK) used a similar approach for the analysis of different tissues (liver, kidney, muscle) and biofluids (blood, milk) from a pod of stranded long-finned pilot whales (*Globicephala melas*). Systematic trends as a function of animal age permitted an enhanced insight into the Hg metabolism of this mammal species.

Invited Lectures

Tuesday Late Morning Invited Lectures

Apr 02, 11:00 - 12:00

IN-08

Nanoparticles in food – an overview

Katrin Loeschner

Technical University of Denmark DTU, National Food Institute, Denmark

Nanotechnology and more particularly nanotechnology-based products and materials have a large potential for providing novel solutions to many of the current challenges facing society such as energy supply and resources efficiency, a clean environment, information and communication, mobility and security, and the efficiency of health-related products [1]. Current applications in the agri/feed/food sector are food

additives and food contact materials, whereas potential future developments are expected in the field of nanocapsules and nanocomposites in applications such as novel foods, food/feed additives, biocides, pesticides and food contact materials [2]. With the increasing use of nanotechnology, there is a need for reliable detection and characterization methods for nanoparticles (NPs) in food.

The talk will give a brief overview on the occurrence of NPs in food, the existing regulations and the most frequently used analytical techniques, including asymmetric flow field-flow fractionation (AF4) hyphenated with inductively coupled plasma-mass spectrometry (ICP-MS), single particle ICP-MS and electron microscopy. Further, general considerations regarding the sample preparation for NPs in food will be presented. Examples of our work include the development and validation of a method for silver NPs in chicken meat based on AF4-ICP-MS [3,4], the detection of NPs by single particle ICP-MS in game meat (lead NPs) [5], candy (titanium dioxide NPs) and noodles (aluminum-containing NPs). Currently, we are working on the development of screening procedures for NPs in food and methods for polymeric NPs ("nanoplastics").

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[2] RIKILT and JRC. Inventory of Nanotechnology applications in the agricultural, feed and food sector. EFSA Support. Publ. EN-621, 1–125 (2014).

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IN-09

Proliposomal encapsulation of resveratrol

Natasa Poklar Ulrih

University of Ljubljana, Biotechnical Faculty, Slovenia

Bioactive compounds (e.g. polyphenols) are considered as valuable components due to their positive effect on health. Polyphenols are often concentrated in leaf tissue, bark layers, flowers and other plant materials. These natural sources of actives became attractive in last decade and the extraction of polyphenols from different plants is still interesting due to many factors that impact final extract composition and phenolic content. These com-

pounds are mainly chemical instable and they are also poorly administrated upon oral consumption. We will present proliposomal encapsulation of resveratrol as well as resveratrol-loaded alginate submicron particles (colloidosomes) prepared by emulsification followed by external gelation. Scanning electron microscopy analysis of the air-dried and freeze-dried particles demonstrated their spherical shape, from 100 nm to 600 nm in size. The interactions between the encapsulated resveratrol and lipids were characterised by fluorescence polarisation measurements, electronic paramagnetic spectrometry and differential scanning calorimetry.

Invited Lectures

Tuesday Invited Lecture at Sečovlje Salt Pens Apr 02, 15:00 - 15:30

IN-10

A scientific view of the Sečovlje Salina

Nives Kovač

National Institute of Biology, Slovenia

Salt marshes represent a very significant and very vulnerable natural resource with a great ecological value for the ecosystem as well they can provide many benefit to humans including the opportunity of production of salt and other products such as a brine, healing mud, food etc.. Today, in many parts of Mediterranean salt marshes (with several Salinas operating since ancient times), the integrated approach to the sustainable conservation and development is promoted. This is also the case for Sečovlje salt pans that are the northernmost salina in the Adriatic Sea. Sečovlje Salina is a part of Sečovlje Salina Nature Park (Piran Bay, northern Adriatic) that includes not less than five important protected Natura 2000 habitat types. Besides immense biodiversity and its ecological importance, it is best known for its cultural heritage including traditional process of salt-making. In crystallisation basins, the salt is still produced obtained manually with a method that is several hundred years' old with the daily gathering of saturated brine on the cultivated microbial mat named petola. Beside salt, the thermal mud and brine are also important products usually used for aesthetic and therapeutic purposes (thermal and wellness tourism). Therefore, knowing and predicting how marshes respond to human and abiotic perturbations is important to their exploitation, preserving and survival. This contribution provides a short review of major results of the interdisciplinary research of physical, chemical, and biological characteristics of Sečovlje Salina sediment.

Invited Lectures

Wednesday Early Morning Invited Lecture Apr 03, 9:00 - 9:30

IN-11

Foodomics: a milestone in food and nutritional studies

Urška Vrhoušek

Fondazione Edmund Mach, Food Quality and Nutrition Department, Italy

Metabolomics is applied to a variety of biological fields from medical science to agriculture. Most of human beneficial properties of plants, be they foods, medical resources, or industrial raw materials, are ascribed to plant metabolites. One of the most important class of compounds are the polyphenols, due to compelling evidences of their beneficial health properties and to their impact on food quality. The complexity and remarkable diversity of polyphenols has challenged the analytical performances of the separation and detection methods in terms of resolving power, selectivity and sensitivity required for the identification and quantification of these compounds in different matrices. Targeted metabolomics is a strategy based on the use of predefined metabolite-specific signals, such as MRM transitions, that can be used to accurately and selectively determine the concentrations of a wide range of known metabolites. A targeted metabolomics method using UPLC/MS/MS system has been developed for the quantification >150 polyphenols and for the quantification of some polyphenols catabolites associated with the consumption of fruits. The validated method was found to be particularly flexible, since it can be easily expanded and adapted to the needs of different experiments. It was successfully applied to the analysis of various fruits and wine, as well as in nutritional studies, providing a valuable tool for the metabolite profiling of both the native compounds present in food and some nutritionally important bioactive catabolites in biofluids and organs.

For a mechanistic understanding of the action of polyphenols in living organisms it is fundamental in nutritional studies to include also untargeted metabolomics, a powerful tool to study processes in organisms and to detect new biologically important biomarkers. In our study, a un-targeted high resolution mass spectrometry-based investigation was chosen to monitor the metabolic effects induced by administration of different dietary polyphenols, at a physiologically relevant dose.

Invited Lectures

**Wednesday Late Morning
Invited Lectures
Apr 03, 10:30 - 11:30**

IN-12

Development of honey, vegetable oil, flour, and collagen stable isotope reference materials

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¹Indiana University, Department of Earth and Atmospheric Sciences, United States

²Fondazione Edmund Mach, Department of Food Quality and Nutrition, Italy

³IAEA Food and Environmental Protection Laboratory

(FEPL), Austria

⁴Jožef Stefan Institute, Department of Environmental Sciences, Slovenia

Stable isotope analysis has been used to detect economically motivated adulteration and substitution of food products since the early 1970s. However, no chemically complex agricultural plant or animal-derived food materials have been offered so far as isotopic reference materials (RMs) by major organizations such as the International Atomic Energy Agency (IAEA) or the National Institute of Standards and Technology (NIST). The fundamental analytical principle of identical treatment of sample and standard advocates chemical similarity of sample and RM, and thus calls for the development of food matrix isotopic RMs. The analytical mandate of two-point normalization along isotopic scales requires at least pairs of isotopically divergent RMs. This presentation describes a current ring-test effort to develop pairs or triplets of (i) honey RMs from tropical Vietnam and Saskatoon in Canada, (ii) flours from C3 and C4 plants, (iii) vegetable oils from C3 and C4 plants, and (iv) collagen powders from marine and terrestrial fauna. Our project places strong emphasis on proper storage of bulk supplies of the future RMs under vacuum or noble gas to exclude oxygen and to extend the shelf life to decades when RMs are stored frozen in the dark. End-users will be able to obtain 0.5 to 1 g or mL aliquots of RMs with recommended isotopic compositions for applications of stable isotope analysis to confirm food authenticity and provenance.

IN-13

The role of research infrastructures in supporting Metrology in Food – the experience of METROFOOD-RI

Claudia Zoani

ENEA - Italian National Agency for New Technologies, Energy and Sustainable Economic, Italy

In the food sector, measurement reliability is a key factor to address technological innovation, sustainability, food safety and security actions. Metrology in Food is an emerging discipline enabling the introduction and spread of metrological principles and concepts into the food & nutrition area. Furthermore, high-quality data on the food chain are of fundamental importance to populate the expanding data technologies with useful contents and, according to the FAIR principles, enable advanced research on food and food metrology. Research infrastructures (RIs) play a central role in the progress and application of knowledge in Europe, and the establishment of a landscape of first-class sustainable RIs and services open to researchers, industry, and other interested groups is of strategic importance in the context of the European Research Area.

METROFOOD-RI (ESFRI Roadmap 2018 – Domain Health and Food) provides high-quality metrology services in food and nutrition, comprising an important cross-section of highly interdisciplinary and interconnected fields throughout the food value chain, including agrifood, sustainable development, food safety, quality, traceability and authenticity, environmental safety, and human

health. It is focused on supporting metrology in food by applying a comprehensive approach to food quality & safety and linking food safety – other than with quality, traceability and authenticity – with nutrition and risk assessment. The RI consists of a physical infrastructure and an electronic one to coordinate and integrate existing networks of plants, laboratories, experimental fields/farms for crop production/animal breeding, small-scale plants for food processing and storage, kitchen-labs for food preparation. The e-RI will make available an access platform to share and integrate knowledge and data on metrological tools for food analysis, focusing on food composition, nutritional contents, levels of contaminants and markers. The scientific offer is addressed to a broad set of users, such as: public and private labs and groups engaged in research activities for food data collection and measurement reliability and basic frontier research in food and nutrition; Food Business Operators and producer associations; policy makers, food inspection and control agencies; consumers/consumer associations and citizens. METROFOOD-RI can allow to strengthen scientific knowledge, promoting cooperation and encouraging the interaction between the various stakeholders and the creation of a common and shared base of data, information and knowledge. Therefore its practical realisation as ready-to-operation infrastructure can represent a strategic and concrete solution to achieve the harmonization, quality and reliability required in metrology in food.

Invited Lectures

Wednesday Afternoon Invited Lecture Apr 03, 12:15 - 12:45

IN-14

Food and nutrition data management and knowledge discovery

Tome Eftimov

Jožef Stefan Institute, Computer Systems Department, Slovenia

Nowadays, there is a high demand for facilitating the Food and Nutrition Sciences with the tools for knowledge discovery maximizing the community engagement. To make this possible, a good food and nutrition data management is a requirement. The data management process should address several questions: i) how to deal with heterogeneous data and ii) how to match and aggregate heterogeneous data that are described and classified in various ways. In order to understand the processes that are crucial for the management, we are going to discuss several use cases, such as information extraction of food- and nutrition-related entities from textual data (i.e. unstructured data) and its harmonization using a unified classification system (i.e. an ontology). Understanding these steps will lead to an efficient data management system that satisfies the requirement for interoperability, which means the ability of data and tools from heterogeneous sources to integrate or work together with a minimal ef-

fort.

Invited Lectures

Wednesday Closing Invited Lecture Apr 03, 13:15 - 13:45

IN-15

A non-analytical solution to supply chain integrity countering fraud

Gerald A. Herrmann

Organic Services GmbH, Germany

Dealing with the issue of food fraud can feel like looking for the needle in the haystack. There are hardly any witnesses as consumers often become victims of the crime unknowingly, and even food companies that buy food products are often unaware of the exact origin and history of the product that they purchase. It is therefore not surprising that when food fraud scandals rocked the food industry (such as the horsemeat scandal), the European competent authorities, law enforcement, and policymakers found themselves staring at the haystack, not knowing where to start.

Check X/ Check Organic® is a cloud-based platform that combines real-time certification with real-time transaction data across the supply chain. It is a solution that can be applied in all commodity-based supply chains. Check X manages data confidentiality through client set-up and a system of rights and roles. Check X uses a traffic light system (red, yellow, and green lights), which was highlighted as intuitive to immediately understand. Single company supply chain solutions as well as system-wide solutions (national, product based, Geographic origin, etc.) based on registration or certification of all system participants are feasible.

Check X is a tested and effective tool for food fraud prevention in the food industry in Europe and beyond in case the following prerequisites are met:

1. Required supply chain actors within the defined system must participate.
2. Production and trade must be recorded/ (estimated).
3. Relevant product qualities must be recorded.

With these prerequisites, the mass balance approach requires much less data than for example a batch traceability approach, is less cumbersome and prone to gaps than other approaches such as internal audits and/ or the building of close business relationships and can prevent fraud from happening rather than detect it once it has happened.

If we return to the metaphor of searching the needle in the haystack, then analytical science and specifically product analysis – by focusing in on understanding the outcomes of fraudulent practices – is a way of understanding the needle. This allows companies to avoid the purchase and trade of such tainted food products. And it allows law enforcement to trace the steps from the needle to the one who committed the fraud. However, if you don't know the character of the needle, the choice of the adequate tool for identifying the needle can be cumbersome, expensive and may fail. Our integrity management solution

Check X/ Check Organic® proposes a different approach: Instead of following the assumption that food fraud is taking place and detecting fraudulent practices as they manifest in the product, food fraud defense programs should use their knowledge to prevent food fraud by comparing produced volumes to traded volumes and preventing food fraud before it has occurred. What we propose is to define the properties of hay, and the size of the hay stack, thus excluding anything that does not fit these definitions, and complement this approach with targeted analytical tools – to identify the needle.

**ORAL
PRESENTATIONS'
ABSTRACTS**

OR-01

State of the art Isotope Ratio Mass Spectrometry (IRMS) applied to food traceability

Lionnel Mounier, Dave Wanless

Thermo Fisher Scientific, France

Several analytical methods, including chromatography, spectroscopy or mass spectrometry, can reveal adulterations or can enable to track the origin of substances. Isotope Ratio Mass Spectrometry (IRMS) is a particularly efficient technique when it becomes necessary to distinguish between the sources (what from) and between the origins (where from).

The isotope ratio fingerprinting is based on the analysis of small isotopic differences in compounds in order to determine the relative isotopic abundances of elements such as C, N, O, H and S contained in the material. The measured isotopic differences are originating from the physical and biochemical isotope fractionation occurring in nature, they are providing information about the source, the origin, and the naturalness of substances. Based on isotopic ratios it is possible to collect information about the geographical, the chemical or the botanical origin of substances and isotopic ratios can enable the differentiation between natural and synthetic products.

The purpose of the presentation is to explore and to illustrate the state of the art in Isotope Ratio Mass Spectrometry to characterize the origin and the naturalness of food products.

Recent advances in instrumentation and in applications will be highlighted.

OR-02

Authentication of the geographical origin of hops (*Humulus lupulus* L.)

Miha Ocvirk¹, Iztok J. Košir¹, Marijan Nečemer², Nives Ogrinc³

¹Slovenian Institute for Hop Research and Brewing, Agrochemistry, Slovenia

²Jožef Stefan Institute, Department of Low and Medium Energy Physics, Slovenia

³Jožef Stefan Institute, Department of Environmental Sciences, Slovenia

Authenticity of the geographical origin of hop plant (*Humulus lupulus* L.) is of paramount importance for brewers, since the contents and compositions of the bitter resins and essential oils in hop plants, which are responsible for beer flavour and aroma, depend on the environmental conditions during its growth. Fraudulent harvest data, such as mislabeling its geographic origin, is becoming an increasing problem globally. A need exists for a reliable method for determining the geographical origin of hops. The set of 77 samples used in this study comprised of hop plant cones from ten main hop growing regions in the World and were analyzed using Isotope Ratio Mass

Spectrometry (IRMS) to obtain $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values and Energy Dispersive X-Ray Fluorescence spectrometry (EDXRF) to obtain the multi-elemental profiles. The $\delta^{15}\text{N}$ (2.2 ‰ to 8.4 ‰) and $\delta^{34}\text{S}$ (0.7 ‰ to 12.3 ‰) values proved to be the most discriminating for classifying hop samples according to geographical origin. ANOVA show distinct groupings for eight out of the ten hop-growing regions. Multi-element profiles proved to be a useful tool in determining the geographic origins of produced hop plants. With EDXRF in combination with discriminant analysis, we were able to completely distinguish between European samples, as well as between non European samples.

OR-03

Comparison of organic and conventional beer in view of the content of the selected phytoestrogens

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¹Jožef Stefan Institute, Department of Environmental Sciences, Slovenia

²Slovenian Institute for Hop Research and Brewing, Agrochemistry, Slovenia

According to theoretical assumptions, organically produced plants synthesize higher amounts of certain secondary metabolites as a response towards pests. There are some studies found in literature, which results support these theoretical assumptions. Prenylflavonoids are secondary metabolites uniquely found in hops. They show antioxidative and weak estrogenic activities. The aim of this study was to investigate if there is a significant difference between the content of the selected prenylflavonoids – xanthohumol, isoxanthohumol, and 8-prenylnaringenin in beers labeled as organic compared to the content in conventional beers.

Beer samples were cleaned by means of solid phase extraction, using Strata-X polymeric reversed phase cartridges, and eluted with acidified methanol. An LC-MS/MS method for the analysis of the three compounds was developed and validated on Shimadzu Nexera X2 UH-PLC coupled to a Sciex 4500 QTRAP system. The compounds were separated on Supelco Ascentis® Express C-18 column (50 x 2.1 mm, 1.7 μm), and eluted by gradient pumping of acetonitrile and 0.1% formic acid in water as mobile phase. The analytes were detected and quantified in multiple reaction method mode, previously optimized for better sensitivity.

The obtained results were statistically processed in order to estimate the difference in analytes' content between the whole groups – organic vs. conventional beers, as well as within the matched pairs – pairs of beers of the same type and very similar alcohol content. Finally, the influence of type of brewing and alcohol percentage on the content of the analytes was also evaluated. The obtained results will be further complemented by the analysis of hops and beer produced from the analyzed hops to confirm the reliability of conclusions and discuss findings.

OR-04

Isotopic and multi-elemental signatures as indicators of origin of sparkling wines

Robin Cellier, Sylvain Bérail, Julien Barre, Ekaterina N. Epova, Olivier F.X. Donard
Université de Pau et des Pays de l'Adour, France

The quality of food is a highly important subject worldwide. Despite of this fact, frauds have always existed especially in the luxury market. In this economic sector, the identification of wine fraud is one of the most important and challenging issues facing the wine industry. Tracing the geographical origin and authenticity of wines allows to detect false description of origin.

In order to determinate Champagne wines authenticities and discriminate from counterfeit products, both multi-elemental analysis and Sr, Pb isotopic ratios were determined in 56 sparkling wines from France, England, Italy, Portugal, California, Australia and Spain. Major (Na, K, Ca and Mg) and trace elements (Li, B, Al, Sc, V, Cr, Mn, Fe, Ni, Cu, Zn, Rb, Sr, Ba, Pb and U) were measured by quadrupole ICP-MS. A methodology for the precise and accurate determination of the Sr and Pb isotopic ratios in sparkling wine were developed using MC-ICP-MS.

These combinations of analysis allowed to separate the Champagne from sparkling wines coming from other countries and address distinctive elements important for the discrimination of wine categories and origins.

Food Authenticity and Traceability

Monday Late Morning Session Apr 01, 11:45 - 12:45

OR-05

Tractability and quality control in commercial truffles (*Tuber* spp.) – a need or an unachievable aim?

Tine Grebenc¹, Lidija Strojnik², Bor Krajnc², Tina Unuk¹, Nives Ogrinc²

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Fungi from the genus *Tuber* (true truffles) are ectomycorrhizal fungi that form sequestrate sporocarps commonly known as truffles. Truffles were of high human interest for millennia all from Persian times till recently as an aromatic delicacy and source of proteins. Truffles have a circumbo-real distribution and comprise around 180 different species among which about 10-12 (ca 6 from Europe, 3 from N. America and 3 from Asia) have significant commercial values ranging from several 10€/kg to several 1000€/kg. Aroma is the main driver of price and market demand and is primarily dependent on truffle species, ripeness, fruiting site and overall quality. High economic value, variable level of knowledge on specific characteristics, and inter-continental differences in species and their distribution are potential reasons for customer confusion, marked frauds and lack of any official quality control on markets. We propose a set of potential analyses that could serve as

a quality control of fresh truffles in the market both, for their identification (species/strain identity and geographic origin) and quality assessment. Based on a reference collection we tested molecular and morphological identification procedure and in parallel an aromatic volatiles and chemical composition analyses to confirm identity and origin of selected truffle species from the designated test area. A geographically well represented reference database as a herbarium voucher collection and a extracted DNA base was built up for most of European countries, with special focus on the SE Europe, and for most of commercial truffle species. *Tuber aestivum* Vitt. and *Tuber magnatum* Pico, as two most commonly traded species in the region were tested for aromas quality, and nutrient and isotopic composition (see Krajnc et al. 2019 and Strojnik et al. 2019 in this publication). With applied approaches were able to clearly separate species and, in some cases, disclose some regionally specific variation. Chemical and aromas analyses showed variations among samples from different locations. The proposed combination of analyses has a potential for reliable tracing of origin and quality of commercial truffle species and should be further developed for its future commercial exploitation.

OR-06

Investigation of truffle aroma compounds: can we differentiate between different species?

Lidija Strojnik¹, Tine Grebenc², Nives Ogrinc¹

¹Jožef Stefan Institute, Department of Environmental Sciences, Slovenia

²Slovenian Forestry institute, Slovenia

Truffles (*Tuber* spp.) are part of the ascomycota phylum of the fungi kingdom, and are found underground, growing as symbionts with specific trees by forming ectomycorrhizae. The fruiting bodies produce hundreds of volatile compounds, which are responsible for the prized characteristic aroma of truffles that show variations between subspecies due to geographical origin, maturity, hydration and storage conditions. The rarity of certain truffle species makes them among the most expensive foods available on the market. The most valuable and costly species are *T. magnatum*, *T. melanosporum* and *T. aestivum*. The truffle *T. magnatum*, better known as “Alba white truffle” can fetch thousands of euros per kilo. A key compound in the aroma of the white truffle is bis(methylthio)methane (also known as 2,4-dithiapentane). This compound is soluble and stable, with highly effective and persistent olfactory characteristics, and because it is inexpensive to synthesise, producers commonly use it to strengthen the aroma of products, made from or in imitation of other less exquisite truffle species, such as *T. melanosporum* and *T. aestivum*, which are characterised by a different set of aromas. Moreover, natural sources of truffle aroma are commonly falsified in terms of dilution, mixing and incorrect declaration of origin. Given the high economic value of certain truffle species, analytical approaches are required to discriminate between natural and synthetic truffle aroma. Currently, gas chromatography coupled to combustion-isotope ratio mass spectrometry (GC-C-IRMS), for deter-

mining the $\delta^{13}\text{C}$ value of the key aroma compounds in food, is a recognized technique for determining authenticity. In the present study, we characterised the aroma profiles of 173 samples of 10 different Tuber species (*T. aestivum*, *T. magnatum*, *T. brumale*, *T. macrosporum*, *T. melanosporum*, *T. excavatum*, *T. mesentericum*, *T. rufum*, *T. indicum*, *T. fulgens*) harvested between August 2018 to February 2019. The majority of samples were collected in Slovenia, Macedonia, Croatia, Italy, Spain and Slovakia. Three samples of *T. indicum* were imported from the Far East. Their aroma profiles were obtained using HS-SPME GC-MS analysis in order to distinguish between different species. Additionally, for confirmation of authenticity, we have developed a $\delta^{13}\text{C}$ database of truffle aroma compounds from well-defined origins, using GC-C-IRMS. We characterised 74 samples of truffles (*T. Aestivum* (52), *T. magnatum* (14) and other species (8)). The results show significant variability in $\delta^{13}\text{C}$ values between different aroma compounds.

OR-07

Towards a multielement and n(87Sr)/n(86Sr) isotope ratio landscape of Austria for food provenancing

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The n(87Sr)/n(86Sr) isotope ratio combined with the multi-element pattern has been identified as powerful tool to retrospectively prove the origin of food. The method is based on the reflection of the local geographical chemical fingerprint of soil and water as fingerprint in different food commodities (vegetables and fruits, fish). The assignment of food products to a certain geographical area can be accomplished via two methods: either by matching the chemical fingerprint a) to a set of reference samples ("database approach") or b) to a map showing the geographical distribution the analysed elements and isotopes ("chemical landscape approach"). The major advantage of using e.g. a distribution map of the 87Sr/86Sr isotope ratios (Sr-isoscape) in soil or water bodies is, that it can be applied independently of the type of plant or fish and can be considered as time-stable (a major advantage in comparison to databases based e.g. on light isotopes of N, O, C). Therefore the spatial distribution of n(87Sr)/n(86Sr) isotope ratios as well as the multielement pattern of the bioavailable fraction in soils and water of the main production areas in Austria were systematically mapped. A spatial database of the determined values together with a statistical assignment tool was developed. Soil, water, fish and vegetable samples were collected systematically across Austria. The samples were analysed using (multi collector) inductively coupled plasma mass spectrometry ((MC) ICP-MS). First results showed a

distinct distribution of the n(87Sr)/n(86Sr) isotope ratios across different regions in Austria. Discriminant analysis showed that by the combination of the n(87Sr)/n(86Sr) isotopic and the Sr, Ca, Mg, Mn, Cu, Zn, Rb, Ba and the Sr/Ca ratio elemental pattern soil samples could be discriminated according to their origin by 100 %. Food commodities like carrots, beets, asparagus, potatoes and pumpkin seeds could be discriminated by these parameters according to federal state, geology, soil type and parental material for soil formation with error rates of only 1-2 %. Fish samples could be also discriminated according to their origin using the n(87Sr)/n(86Sr) isotope ratio as primary parameter. Future work will increase the spatial coverage of the agricultural and aquatic production areas.

Acknowledgements: COMET-K1 competence centre FFoQSI, funded by the Austrian ministries BMVIT, BMDW and the Austrian provinces Niederoesterreich, Upper Austria and Vienna within the FFG COMET programme.

OR-08

Geographic traceability of North Italian apples based on Sr isotope ratio and multi-chemical fingerprint

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Italy is the second largest apple producer in the European Union (EU-28), behind Poland, with an average quantity of harvested apples higher than 2.4 million Mg (2014-2016). Although its acreage has decreased in the recent years, Golden Delicious is the variety mostly spread on a national scale. Three districts in North Italy are particularly famous for its cultivation: Val di Non and South Tyrol in Trentino Alto Adige and Valtellina in Lombardy. Here, several aspects, including long agricultural traditions, human factors, mountain environments, and peculiar climatic conditions, favoured the cultivation of apples, whose quality has been officially recognized through the EU quality labels. Since the 2000s, Golden Delicious apples from Val di Non awarded the PDO (protected designation of origin) label and those from South Tyrol and Valtellina the PGI (protected geographical indication) label.

With this study, we tested the use of the strontium isotope (87Sr/86Sr) ratio, a soil-derived traceability marker that varies according to geology, to classify apples based on their provenance. Apples (cv. Golden Delicious) were collected from the PDO and PGI cultivation districts (Val di Non, South Tyrol, and Valtellina) and from other districts of North Italy. In total, the sampling was performed in 41 orchards at 2017 harvest. In half of the orchards, the sampling was repeated also the following year, collecting soil and apple samples. A good correlation ($R > 0.98$)

was found between the apple and the soil $87\text{Sr}/86\text{Sr}$ ratios, with the latter depending on the local geological features. On average, no significant difference was measured between the results of the $87\text{Sr}/86\text{Sr}$ ratios of apples collected in 2017 and 2018, confirming that the $87\text{Sr}/86\text{Sr}$ ratio does not significantly vary from year to year. However, the results of the $87\text{Sr}/86\text{Sr}$ ratio analysis did not allow a complete separation of apples according to their provenance, as the soil from some areas had similar $87\text{Sr}/86\text{Sr}$ ratios. To improve the output of the study, a multivariate approach was tested combining the $87\text{Sr}/86\text{Sr}$ ratio analysis with the multielement analysis. A classification model based on the linear discriminant analysis (LDA) was developed, with satisfying preliminary results (accuracy of 88-78% in calibration and prediction, respectively). This study represents a first effort to enhance the tutelage of high-quality apples cultivated in the Italian districts using objective and reliable analytical tools and a good starting point for future works.

Food Authenticity and Traceability

Monday After-lunch Session Apr 01, 15:00 - 15:30

OR-09

Elemental content and strontium isotope characterisation of Slovenian wine

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Slovenia has 15,800 hectares of vineyard area divided into three major grape-growing regions, Podravje, Posavje and Primorska, which are further divided into 9 sub-regions. The composition of wine is influenced by the characteristics of the production areas, such as grape variety, soil and climate, as well as winemaking process and wine storage. The elemental compositions of wine as well as the isotopic compositions of each element may be influenced by several process; the uptake of minerals from soil, which can be of both natural and anthropogenic origin (i.e. the use of fertilizers and pesticides), and by the winemaking processes, such as the use of various additives.

In the presentation the first data on the characterisation of the Slovenian wines will be presented. Namely, the results of multi-elemental and Sr isotope compositions determination in wines from three main Slovenian wine production regions. Two wine grape varieties were analysed; white wine, the Pinot (gris or/and blanc), and the red wine, Merlot. The aim was to evaluate the differences in elemental composition and Sr isotope ratio of the same wine variety growing in different regions in order to find the characteristic tracers for the investigated wine production areas. Major and trace elements, rear earth elements and $87\text{Sr}/86\text{Sr}$ isotope ratio were determined in total of 10 wine samples from Podravje (Štajerska sub-region), Posavje (Bizeljsko-

Sremič sub-region) and Primorska (Goriška brda, Slovenian Istria and Vipavska valley sub-regions). Cr was elevated in wine samples from Primorska region, most probably as a consequence of the flysch bedrock that is rich in Cr, and was an important discriminator for the wines from Primorska region. The $87\text{Sr}/86\text{Sr}$ isotope ratio ranged from 0.708715 to 0.71420, with the highest values found in the Podravje region, which was therefore the important discriminator for this region.

Although Slovenia is a small country, it is geologically very heterogeneous. On its approx. 20.000 km² various types of bedrocks exist, thus reflecting in different mineral composition and $87\text{Sr}/86\text{Sr}$ signatures. The results of the present study confirm that by the use of multi-elemental and Sr isotope ration analysis combined with the statistical analysis it is possible to find characteristic tracers for the different wine production regions, even though they are not far away between each other.

OR-10

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ analyses of amino acids of grape

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$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of free amino acids in grape reflect the source of C and N of grapevine and the metabolic pathways involved in their biosynthesis. In this study, carbon and nitrogen isotope ratio analysis of whole amino acids extracted from several Italian musts and wine through ion-exchange chromatography and of proline after protein hydrolysis and derivatization were carried out by elemental-analyse- (EA) and by compound-specific gas chromatography-combustion- (GC-C) coupled with an isotope ratio mass spectrometry (IRMS). The sugar fraction isolated from must was also analysed. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of amino acids are not correlated. Our results showed a good correlation between the $\delta^{13}\text{C}$ of sugar and amino acid fractions. This could be effective in improving detection of the illegal addition of exogenous sugar to grape must. Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of amino acids of must are related with those of wine. The $\delta^{15}\text{N}$ of amino acid seems to be related with the isotopic composition of soil, agricultural practices and with the climatic condition during ripening.

We concluded that stable isotope ratio analysis of amino acids represents a novel analytical tool to support and improve certification and control procedures.

Food Quality and Safety

Monday Afternoon Session Apr 01, 16:30 - 18:00

OR-11

Human biomonitoring and its role in food safety: trace elements in men and lactating women living in Slovenia

Janja Snoj Tratnik¹, Ingrid Falnoga¹, Darja Mazej¹, David Kocman¹, Vesna Fajon¹, Marta Jagodic¹, Anja Stajniko¹, Ajda Trdin¹, Zdenka Šlejkovec¹, Zvonka Jeran¹, Joško Osredkar², Alenka Sešek-Briški², Mladen Krsnik², Alfred B. Kobal², Lijana Kononenko³, Milena Horvat¹

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The first national human biomonitoring in Slovenia surveyed cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As), manganese (Mn), selenium (Se), copper (Cu) and zinc (Zn) in a childbearing population (18–49 years) selected from lactating primiparous women and men (N = 1084). The overall aim was to estimate trace elements' levels and geographical variations in order to identify sources of possible exposures and set the national reference values. The study population was selected evenly from 12 study areas across Slovenia, including rural, urban and known or potentially contaminated environments. Within 6–8 weeks after delivery, venous blood, spot urine, scalp hair and breast milk samples were collected to determine the selected elements. The data analysis included descriptive statistics and multiple linear regression using elemental concentrations in biological matrices, questionnaire data and environmental datasets. Essential elements showed no significant deficiencies or excessive levels in the study population and were largely determined by sex and/or the participating women's physiological status (postpartum, lactation), as well as by certain dietary sources. Toxic elements' levels were mainly below the levels considered to present increased health risk. Nutritional and other life-style habits appeared as significant determinants of exposure to Cd (game meat consumption and smoking), Hg (seafood consumption and amalgam fillings), As (seafood consumption) and Pb (alcohol consumption, smoking, game meat consumption and type of water supply). Increased seafood consumption in the coastal study area contributed to higher Hg and As (arsenobetaine) levels. A distinctive geographical pattern was also confirmed, due to past mining activities combined with naturally elevated background levels in the cases of Pb (Mežica Valley), Hg (Idrija and Posočje) and As exposure (Zasavje). Extensive sample size database accompanied with life-style (including nutritional) and environmental data improved the prediction of exposure patterns. Furthermore, the country-wide coverage enabled setting the reference values for the childbearing population living in Slovenia, being the first study to establish reference values specifically for lactating primiparous women. Based on these results, recommendations for consumption of certain food items, particularly for the sensitive population group of pregnant or lactating women, can now be prepared.

OR-12

Diet related exposure to As in Slovenian-Croatian women population (PHIME-CROME study)

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Arsenic (As) is potentially toxic metalloid to which humans are mainly exposed through food consumption - primarily seafood, and through drinking and cooking water. Present study evaluated As exposure and As detoxification efficiency in Slovenian-Croatian women by following As in blood and urine and urine As metabolites (arsenobetaine - AsB, inorganic arsenic - iAs, mono- and di-methylated As forms - MMA and DMA).

Population consisted of 136 pregnant women from coastal region of Rijeka (aged 20 – 44 years) and 176 non-pregnant women from city of Ljubljana (aged 30 -51 years). Geometrical mean of As levels were 2.6 ng/ml in blood and 23 ng/mL in urine. More than half of the As in urine was in form of biologically inactive AsB, while the sum of biologically active metabolites (iAs, MMA and DMA) was as expected very low; 3.2 ng/mL. In general, As levels were significantly higher among pregnant women, residents of coastal area with higher seafood intake frequencies. Consequently, pregnant women had higher share of As in form of AsB (arithmetic mean: 78%) than non-pregnant women from central part of Slovenia (arithmetic mean: 47%). Additionally, they have higher level and percentage of DMA. This points on their more efficient methylation, which is important as detoxification mechanism of iAs. That means that physiological changes during pregnancy are affecting As metabolism in protective way.

Taken together, As exposure did not present a threat for studied women, as they were exposed mostly to the non-toxic arsenobetaine, while the exposure to biologically active iAs was very low. Nevertheless, the assessment of As methylation efficiency at different physiological states, such as pregnancy, is important for the risk assessments at higher iAs exposures. Moreover, the influence of individual genetic variations in arsenic (AsIII) methyltransferase (AS3MT) - responsible for As methylation - should be considered.

OR-13

Determination of neonicotinoid pesticides in Slovenian honey

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Neonicotinoid pesticides are a relatively novel group of insecticides with low toxicity towards non-target species and good environmental degradability. In the recent years, they have been extensively investigated due to an apparent link to colony collapse disorder in bees. The risk was confirmed in 2018 by the European Food Safety Authority. The use of three neonicotinoids: clothianidin, imidacloprid, and thiamethoxam was temporarily banned by the European Commission in 2013 and further restricted in May 2018. Slovenia has already in 2011 imposed a moratorium on the same neonicotinoids but three years later, clothianidin residues were still present in dead bees. There has been no country-wide study looking at the effectiveness of the ban or levels of those neonicotinoids that are still in use. Honey analysis is a non-invasive way of monitoring the exposure of bees to neonicotinoids. Besides the three restricted compounds, two additional neonicotinoids registered in Slovenia: acetamiprid and thiacloprid, were included in the study.

Two sample preparation methods: solid phase extraction (SPE) and QuEChERS were optimized for the preconcentration of trace residues of neonicotinoids from honey. Extracts were analysed by the newly developed LC-MS/MS method. After optimal parameters were established, both extraction methods were fully validated at two concentration levels, according to typical maximum residue levels (MRL) for neonicotinoids in honey. Furthermore, they were compared in terms of validation parameters and their compatibility with LC-MS/MS. For each of them, calculation of uncertainty of determination was made according to SANTE/11945/2015 protocol.

Both methods gave satisfactory recoveries, precision, low limits of detection and quantification, and low combined uncertainty of determination. In comparison, QuEChERS showed slightly better accuracy and lower matrix effect than SPE. It was therefore applied to the analysis of 51 honey samples collected in 2014-2016 from the whole area of Slovenia.

Low contamination below MRL with acetamiprid and thiacloprid was detected in the Slovenian honey. The current ban was implemented in 2011 and the samples were collected three to five years later. The three banned neonicotinoids were below the LODs in all the samples, but bees remain exposed to the registered ones. This survey of Slovenian honey contributes to the global database of exposure of bees to the neonicotinoid pesticides.

OR-14

Ongoing work on implementing cumulative exposure assessment to determine chemical food safety in Slovenia

Urška Blaznik

National Institute of Public Health, Slovenia

At present, regulation and risk/exposure assessment of chemical compounds in food (pesticide residues, food additives, contaminants, ...) is based on adverse health effects of single compounds. However, real life can be more

complex as chemical exposure may occur through multiple sources and sequential exposures. Combined exposures potentially can lead to cumulative effects.

Current status. Although the need to address combined exposures to mixtures of substances and their combined risk is included in current legislation, no validated strategy is yet available. This was identified as a problem by various international bodies involved in public health, including the European Food Safety Agency (EFSA).

EFSA adopted generally accepted principles related to cumulative risk assessment methods and further proposed a system of grouping substances that produce common adverse outcomes in the same target organ/system into cumulative assessment groups. The pilot assessments looking at the cumulative effects of exposure to pesticides in food on the human nervous and thyroid systems are expected to be finalised by the end of this year.

EFSA's methodology is being implemented in the Horizon 2020 project Euromix (European Test and Risk Assessment Strategies for Mixtures) coordinated by RIVM, Netherlands. NIJZ (Slovenia) contribute to the Aggregate and cumulative exposure assessment-working group. The EuroMix approach for mixture risk assessment is component-based. Substances are grouped based on a common toxicity. Hazard and exposure information for each substance in the assessment group is used for estimation of the combined risk using the dose-addition methodology and relative potency factors. The focus is on dietary exposure but other exposure routes are also addressed.

Main challenges. Probabilistic modelling of the dietary exposure requires detailed individual based consumption data and measured concentration data for substances that are included in the assessment. In 2019, the Slovenian Dietary survey "SI.Menu 2017/18" will be finished and for the first time provide detailed information on food consumption of the Slovenian population from 3 months to 74 years old. The dietary study was especially needed for infants and toddlers, since no national food consumption data for this population group was yet available. The concentration data is a great challenge for Slovenia, which is a small country.

Future work. The outcomes of the first assessments by EFSA and experiences gained in the EuroMix project will form the basis for further implementation of mixtures exposure assessment.

OR-15

Novel molecular approaches for quantification of genetically modified organisms

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Since their introduction on the world market more than 20 years ago, genetically modified organisms (GMOs) have been adopted by many countries. Genetically modified crops, ornamental plants, trees, and even fish, carrying different new trades, from herbicide tolerance and insect resistance to altered growth patterns and abiotic stress (e.g. salinity) resistance, have been developed.

With the increased use of GMOs, the majority of coun-

tries, including the European Union (EU), implemented a labelling system for products containing GMOs based on a certain threshold. In the EU, the labelling threshold for food and feed products that contain, consist of, or are produced from authorised GMOs is set at 0.9% per ingredient. A zero tolerance level is set for unauthorised GMOs. Therefore, to control the presence and quantity of GMOs in the food and feed supply chains, sensitive and specific detection and quantification approaches are needed.

As GMOs express at least one additional trait encoded by one or more genes, introduced into the organism by means of recombinant DNA technology, which differentiates the GMO from the wild-type, GMO testing is based either on detection of recombinant DNA or on detection of the recombinant protein encoded by the inserted gene. Today, DNA-based approaches are most commonly used for GMO quantification, with real-time quantitative polymerase chain reaction (qPCR) as the standard method. However, with the rise in the number of GMOs and products containing GMOs, qPCR is no longer time- and cost-efficient. Additionally qPCR is prone to inhibitions, and with the increasing complexity of the products, the possibility of inhibition is also on the rise. A new approach is needed. From most notable novel approaches such as digital PCR (dPCR), loop-mediated isothermal amplification (LAMP), PCR capillary gel electrophoresis, and microarray and Luminex technologies, only dPCR enables quantification of DNA.

To overcome the above stressed issues of qPCR we have used three dPCR platforms, BioRad - QX100/200, Fluidigm - Biomark, Stilla - Naica in our laboratory in order to study the use of dPCR for GMO quantification. Our aim was to transfer qPCR methods to dPCR and to design and evaluate multitarget approaches for more time- and cost-effective quantification of GMOs in complex samples exhibiting high inhibition.

OR-16

Exposure to endocrine disrupting chemicals via food: Urinary phthalate metabolites in the Slovenian general population

Agneta Runkel, Darja Mazej, Janja Snoj Tratnik, Milena Horvat

Jozef Stefan Institute, Department of Environmental Sciences, Slovenia

For decades, phthalates are ubiquitously present, non-persistent contaminants in the environment. They are particularly infamous for their role as endocrine disrupters and have been linked to worsening metrics of male fertility, adverse child neurodevelopment, cancer, and other health impacts. Phthalate metabolites are regularly detected in urine in the general population and food is widely accepted as the main exposure route. In 1999, certain phthalates were restricted in children's toys for the first time. In 2015, the European Union further restrained the use of selected phthalates for production within the EU. However, they may still be present in imported products and, thus, persist as a health risk in the environment.

Human biomonitoring is an established tool for the assessment of human exposure to chemicals that yields informa-

tion about exposure levels, routes, and possible associations.

In this study, urine samples of 153 mothers (age 30-52 years), 153 children (age 6-12 years) and 70 men (age 30 - 70 years) were analysed for 7 phthalate metabolites (MEHP, 5OH-MEHP, 5oxo-MEHP, MEP, MBzP, MiBP and MnBP) within the European project DEMOCOPHES. Concentrations in children's urine are significantly higher - with the exception of MnBP and MEP - compared to adults' urine ($P < 0.004$). In all populations, DEHP and DEP metabolites are higher in samples from rural compared urban areas ($p < 0.03$), while MnBP levels show the opposite trend in mothers and men ($p < 0.007$). MiBP levels are (non-)significantly higher in children, mothers and men ($p = 0.02$) from urban areas. Contradictory to the literature, we observed higher urinary levels of MEHP ($p = 0.008$) in older children (age 10 -12 years) compared to younger children (age 6-9 years). DEHP metabolite levels differ significantly between younger (age 30-36 years) and older women (age 37-52 years) ($p < 0.007$), and (among women) significant associations were found between certain phthalate metabolites in urine and consumption of frozen (MiBP, $p = 0.04$) and canned food (MEP, $p = 0.002$). Additionally, associations were found between metabolites and indoor PVC flooring ($P = 0.01, 0.04$ and 0.02 for 5OH-MEHP, 5oxo-MEHP and MBzP, respectively) and smoking habits (MEP, $p = 0.01$; MnBP, $p = 0.02$). For men and children such associations with potential exposure sources still have to be investigated. All associations will furthermore be tested for potential confounders using linear regression modelling.

Food Quality and Safety

Tuesday Early Morning Lectures

Apr 02, 9:30 - 10:30

OR-17

Analytical procedures for speciation of zinc, nickel and chromium in food samples

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In this work, we present the cases in selected speciation analysis.

Human milk is usually the first diet for the infant. It meets all the needs of the new-born and provides essential trace elements like zinc (Zn). The importance of Zn for normal growth and development of infant has been well documented. It is important to know in which highly bioavailable form Zn is present in human milk. By the use of weak anion-exchange CIM DEAE monolithic column, difficulties, like preserving the integrity of moderate stable Zn complexes during analysis and unwanted Zn interaction with various chromatographic column or numerous buffer solutions were efficiently solved in speciation of Zn

in human milk. Separated Zn species were quantified by ICP-MS, while ES-MS-MS was used for ligand identification. Zn-citrate was found to be the only low molecular mass Zn species present in human milk.

Nickel (Ni) is highly allergic to sensitised individuals. Normally, it is present at trace amounts in foodstuffs. So far, little is known about chemical speciation of Ni in food. By hyphenation of CIM DEAE monolithic column with ICP-MS and using ES-MS-MS for ligand identification, it was experimentally found that Ni in various tea infusions exists in a form of Ni-quininate complexes or ionic divalent form of Ni. As a product of the glucose fermentation, gluconic acid was identified as the most important LMM-Ni binding ligand in cocoa infusions. Ni²⁺ and, in most of samples analysed, traces of Ni-citrate were also identified in the cocoa infusions.

Recently, the presence of highly toxic hexavalent chromium (Cr(VI)) was reported in tea infusions and bread. These statements were made without applying any speciation analysis. To confirm that the reported data are an artefact of wrongly applied analytical methodology, Cr speciation was investigated by HPLC-ICP-MS. By the use of ⁵⁰Cr(VI) and ⁵³Cr(III) stable isotopes and speciation analysis it was demonstrated that in samples investigated Cr(VI) concentrations were below the LOD (0.033 ng Cr(VI)/mL). In tea infusions ⁵⁰Cr(VI) was almost completely reduced due to the presence of antioxidants, while the high content of organic matter in bread appreciably reduced ⁵⁰Cr(VI) even in highly alkaline (pH 12) bread extracts.

OR-18

On the toxicity of edible mushrooms – Hg and Se accumulation and bioavailability

Katarina Vogel-Mikuš¹, Anja Kavčič¹, Johannes Teun van Elteren², Marta Debeljak², Iztok Arčon³, Alojz Kodre⁴

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Boletus edulis (King Bolete) and *Scutiger* (*Albatrellus*) *pes caprae* (Goat's foot) are edible mushrooms which form mycorrhizal associations with hardwoods and conifers. They are known Se- and Hg-accumulators and both elements are known to accumulate in caps of fruiting bodies, mostly in inorganic form (more than 80% and up to 99%) (Slejkovec et al. 2000; Miklavčič et al. 2013). The bioavailability and toxicity of Hg and Se from the fruiting bodies to higher trophic levels, however, remain largely unknown.

We determined the localization of Hg and Se in fruiting bodies (the caps) of *B. edulis* and *S. pes caprae* (collected in Idrija and various locations across Slovenia) using imaging methods such as Laser Ablation-Inductively Coupled Plasma-Mass Spectroscopy (LA-ICP-MS) and Synchrotron-X-ray Fluorescence (SR-XRF), while Hg and Se speciation and the ligand environment was determined by X-ray Absorption Spectroscopy (XAS).

Results show that Hg and Se mainly accumulate in the

spore forming part of the mushroom caps, with Hg bound mainly to sulphur compounds, and Se found in the form of selenate and seleno-methionine. We also studied the bioavailability and toxicity of Hg and Se by feeding mushrooms to slugs (*Arion* spp.) and monitoring Hg and Se uptake and lipid peroxidation levels in the slugs' digestive gland and muscles. Feeding mushrooms with high Hg levels to the slugs induced lipid peroxidation in the digestive gland, suggesting that the mushrooms contained distinct amounts of Hg in bioavailable form. Thus, the allowed daily intake of Hg for humans may already be reached at moderate mushroom consumption. Increased Se levels in mushrooms mitigated Hg toxicity in snails; however, the formation of HgSe complexes in the slugs' digestive gland was not confirmed.

References:

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Acknowledgments:

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OR-19

Contrasting allocation of magnesium and calcium in tea leaves may explain their different extraction efficiency into the infusion

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Beside coffee, tea is the most consumed hot beverage in the world. The metabolic composition of tea (*Camellia sinensis* (L.) Kuntze) plant leaves determines the taste and quality of the drink and is prerequisite for consumer acceptance. Much less is known about the connection between elemental composition of tea leaves and the elemental composition of tea infusion. Being aluminium (Al) accumulator, leaves of tea plants contain large amount of Al. Luckily, extraction of Al into infusion has been shown to be «1%. By contrast, on average 13% and 31% of total leaf magnesium (Mg) and calcium (Ca), respectively, have been measured in different tea infusions. While we know that Al is accumulated in cell walls of cells in the

outermost tissues of leaves, epidermis, the distribution of other biologically relevant elements (sodium (Na), Mg, phosphorus (P), sulphur (S), chlorine (Cl), Ca, potassium (K), manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn) at tissue and cell levels in tea leaves has not been comprehensively studied so far. Therefore, we employed micro-particle induced X-ray emission, a technique enabling quantitative and non-destructive mapping of element distribution with superb sensitivity (between <1 and 10 mg kg⁻¹ dry weight) and lateral resolution below 1 µm. Old and mature leaves of plants supplemented with 200 µM Al were evaluated. Resulting distribution maps revealed distinct tissue-specific distribution of all elements studied. The largest concentrations of Mg, S and Mn were found within epidermal cells. Apparent Ca hotspots were observed, which were assigned to Ca crystals, in agreement with a previous observation. Interestingly, significant amounts of Mn, Fe, Cu and Zn co-localised with some of these crystals, particularly in old leaves. Contrasting allocation of Mg and Ca in tea leaves may explain their different extraction into the infusion, although chemical environment of each of these elements will also play a role. Distribution of elements in tea leaves will be presented and their significance for the extraction into the infusion will be discussed.

OR-20

Transfer of potentially toxic elements to Chinese cabbage from soil amended with compost derived from the sewage sludge

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The agricultural utilization of sewage sludge is currently one of the available options for sludge treatment and disposal in the EU. Sludge application to soil is efficient in recycling valuable components such as organic matter, N and P. However, soil amendment with sewage sludge can influence the accumulation of potentially toxic elements (PTE) in soil and consequently there is a risk of phytoaccumulation of PTE. To assess the hazard related to accumulation of PTE by edible plants, the transfer of Cd, Cr, Cu, Pb, Ni, Zn, U and Th to Chinese cabbage grown in soil amended with composted sewage sludge was investigated. Test plants were grown under greenhouse conditions in pots filled with soils amended with different rates of compost. For substrate and plants, the total element concentrations were measured and soil-plant concentration ratios determined. Soil characteristics were assayed, since they directly affect bioavailability of PTE. Concentrations of Cd, Cr, Cu, Ni, Zn, U and Th in cabbage leaves varied from 0.20–0.37, 0.09–4.26, 9.90–189.76, 1.14–2.12, 26.50–61.90, 0.02–0.10 and 0.01–0.68 mg kg⁻¹ dry weight, respectively. Data obtained revealed that phytoaccumulation of Zn and Cu was significantly affected by

compost amendment to soil. Soil-plant transfer of Cd, Pb and U was negligible as their concentration levels in plants were near the limit of detection. The relationships between the PTE amounts in plants and soil characteristics are also shown and the concentration ratios are discussed.

Food Quality and Safety

Tuesday Late Morning Lectures Apr 02, 12:00 - 13:00

OR-21

Optical chemical sensors for food quality

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The deterioration of foods which occurs progressively during storage may result from physical or chemical changes in the food itself or from the activity of micro-organisms growing in, or on, the product. Traditional microbiological methods to determine the extent of deterioration are limited by the time required to obtain results and indirect methods, relying on chemical, physical or physico-chemical changes, often do not give a response until large numbers of cells are present. Even many recently devised rapid methods require significant growth before a detectable response is achieved, whilst others depend on the use of sophisticated and expensive equipment. One of the drawbacks of widely used microbiological methods is that spoilage is not always of microbial origin. Recent trends in food quality and safety promote an increasing search for trace compounds that can affect human health. There are two reasons for the determination of biogenic amines in food: potential toxicity and the possibility of using them as food quality markers. We designed fluorometric method for the determination of biogenic amine agmatine based on SiO₂-SH particles synthesized via the Stöber method under alkaline conditions based on the alkoxide precursors tetraethoxysilane (TEOS) and mercaptopropyl-trimethoxysilane (MPTMS). The particles were characterized based on the transmission and scanning electron microscopy. The success of binding of mercapto (–SH) groups was confirmed by FT-IR analysis, whereas the potentiometric titration provided the information on the amount of –SH, as well as on the amount of silanol (Si–OH) groups. The SiO₂-SH particles, with the molar ratio between the two precursors (P) TEOS : MPTMS = 2:1 (Figure 1), were used as the basis for the bonding of OPA reagent. SiO₂-SH-OPA particles were tested in the presence of various biogenic amines via fluorescence measurements. In this case, we studied the spectral characteristics, the effect of pH and the sensitivity to other biogenic amines. SiO₂-SH-OPA particles showed the highest fluorescence signal change in the presence of AgmS, with a limit of detection of 7.3×10^{-7} M and a response time of 2 min. Furthermore, some real measurements of freshness of the food will be presented.

OR-22

Exposure to nano TiO₂ particles from food contact quasi-ceramic coatings

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Novel food contact materials containing nanoparticles have been developed recently. These novel materials are mostly in the research and development stage, but some of them already reached the market. There is possible risk of consumer exposure to nanoparticles through potential release of nanoparticles from such materials into food.

Example of food contact materials containing TiO₂ nanoparticles that can be found on the market are pans with quasi-ceramic coatings. During the use, matrix of such coatings may be damaged and consequently TiO₂ nanoparticles may be released into food. Damaged surface can be well observed in high resolution SEM images of quasi-ceramic coating used in the kitchen for two years, carefully maintained and handled with no use of sharp objects and dishwasher machine. The quantity of nano TiO₂ released in debris from the surface into food was estimated from the image of used coating taking into account previous characterisation results. Exposure to TiO₂ particles released was assessed on the basis of worst case scenario, for adults and toddlers. Exposure estimated from the image is comparable to estimated dietary intake of TiO₂ nanoparticles from food.

OR-23

Cattle fattening for top quality beef production

Matjaž Červek

Emona Nutrition R&D Dep., Slovenia

Cattle fattening in Slovenia is the second priority behind the milk production in cattle breeding. But we are less efficient on this field in comparison with our north and west neighbour countries. The aim of the project is to prepare the technology of calfs and young cattle (up to 24 months) fattening for the best quality beef production. The technology will be the base to set up Slovenian beef trademark. With it we expect to improve economy of cattle fattening and diminish greenhouse gases production. Feeding tests on calfs and young cattle for top quality beef production will perform on seven farms at different parts of Slovenia. The management and animals nutrition, fattening economy, before slaughter and slaughter procedures and postslaughter technologies will be led by experts from Biotechnical Faculty University of Ljubljana, Agricultural Institute of Slovenia and Emona Nutrition R&D Department. Experts for agricultural economics will do calculations and give guidelines for economical beef production. In the results dissemination will be employed all project partners led by Chamber of Agriculture and Forestry of Slovenia District Subsidiarie Novo mesto.

OR-24

Interactive effect of gamma radiation and salicylic acid on sprout inhibition of two processing potato varieties

Hind Elbashir

University of Bisha, Food Science and Nutrition, Saudi Arabia

The effect of salicylic acid (4000/ppm) and gamma radiation (0.10 kGy) treatments on storage and processing quality of Diamant and Sinora potato tubers (*Solanum tuberosum* L.) were investigated. Potato tubers were tested during cold storage at 10 °C and 73-78% RH. for length of sprouts, fresh weight loss, dry matter content, reducing sugars and chips yield. Tubers were treated with salicylic acid in the field that exhibited fast sprout growth, high fresh weight loss and high sugar accumulation. Spraying with salicylic acid in the field followed by exposure to 0.10 kGy dose gamma rays succeeded in controlling the sprouts till the fourth month of storage period. It was obvious that the application of irradiation doses of 0.10 kGy was effective in checking the break of dormancy, fast sprout growth and high fresh weight loss. Such treatments resulted in acceptable potato products without adverse effect on reducing sugars, dry matter and chips yield of the tubers. After storage for six months, Diamant potato variety was still suitable for making chips and French fries.

Foodomics

**Wednesday Early Morning
Lectures
Apr 03, 9:30 - 10:00**

OR-25

Ultra-rapid determination of tyrosol and hydroxytyrosol derivatives in extra virgin olive oil by paper spray tandem mass spectrometry

Lucia Bartella, Fabio Mazzotti, Iole Gabriele, Giovanni Sindona, Leonardo Di Donna

Università della Calabria, Dipartimento di Chimica e Tecnologie Chimiche, Italy

Extra virgin olive oil is a typical food of the Mediterranean diet. It possess several beneficial effects on human health, widely described in literature. These effects are due to its particular molecular composition; it is, in fact, particularly rich in triacylglycerols containing monounsaturated omega-9 fatty acids. On the other hand, its main effect on human health is related to the presence of radical scavenging molecular species such as tocopherols and phenols. The latter compounds are mainly derivatives of two phenylethanols called Tyrosol (2-(4'-hydroxyphenyl) ethanol, Tyr) and Hydroxytyrosol (2-(3',4'-dihydroxyphenyl) ethanol, Htyr). Recently, the European Commission issued the regulation 432/2012 listing a series of molecules which may provide positive effects on human health; the regulation allows to report on the label the beneficial effects exerted by the food which contains a certain amount of those molecules. In the case of olive oil, the regulation states that: "Olive oil polyphenols contribute to the protection of blood lipids from ox-

idative stress” and “that the beneficial effect is obtained with a daily intake of 20 g of olive oil...which contains at least 5 mg of hydroxytyrosol and its derivatives (e.g. oleuropein complex and tyrosol) per 20 g of olive oil”. It is important, hence, to develop a reliable analytical method, which is able to certify accurately the amount of Tyr and Htyr species in olive oil. Here we present a fast analytical method based on paper spray mass spectrometry to assay Tyr and Htyr derivatives. In particular, the analysis regards both the unbound molecules and their ester conjugates; the latter are submitted to a fast microwave acid hydrolysis step, and the total amount of released Tyr and Htyr is then measured by mass spectrometry. The analysis relies on the use of deuterated tyrosol and hydroxytyrosol internal standards, which allow to achieve the best performance in accuracy and reproducibility. Moreover, SPE has been used for the extraction procedure, in order to minimize the matrix effect, which is a known weakness of ambient mass spectrometry. The accuracy of the measurements, calculated at nearly 100%, was evaluated by analyzing artificially fortified seeds oil samples; then the analytical procedure has been tested on five samples of extra virgin olive oil. LOQ and LOD parameters and the recovery values of the extraction process were also evaluated.

OR-26

Combined metabolic, elemental and isotopic fingerprinting for origin determination of carrots

Zora Jandrić¹, Andreas Zitek², Tim Causon¹, Václav Štursa³, Anastassiya Tchaikovsky¹, Thomas Prohaska⁴, Stephan Hann¹

¹University of Natural Resources and Life Sciences Vienna (BOKU), Department of Chemistry, Austria

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³University of Technology, Faculty of Chemistry, Department of Food Chemistry and Biotechnology, Czech Republic

⁴Montanuniversität Leoben, General and Analytical Chemistry, Austria

An increasing consumer interest in high quality food products with a clear geographical origin has emerged due to globalization of food supply chains and a subsequent increase in the variety and availability of food products from other countries. Verifying the authenticity and origin of foodstuffs is therefore important for the integrity of the whole food supply chain from producer to consumer. Determination of the origin of food is a complex analytical task that requires consideration of the final product in its entirety. The different geographical provenance or different processing approaches of food influence a variety of chemical parameters, which, in their entirety, provide substantial complementary information. Such a multi-chemical fingerprint has the potential as reliable and robust tool to proof provenance and authenticity. Austria's gourmet regions (“Genussregionen”) are culinary regions that boast a unique interplay of local food traditions. Products from these regions guarantee a traceable origin, absolute safety and high quality standards. The carrot (*Daucus carota* L.) is one of the most

economically important vegetables grown in Austria. The potential of applying multivariate data analysis to various chemical parameters (⁸⁷Sr/⁸⁶Sr isotopes ratio, multi-element analysis, and untargeted metabolomics) to provide a unique fingerprint of carrots from specific regions was explored. This approach was applied for the assessment of authentic carrots directly received from producers in five different regions within Austria (Vienna, Tyrol, Salzburg, Vorarlberg, and Upper Austria).

Results from the ⁸⁷Sr/⁸⁶Sr isotopes analysis, multi-element profiling, and untargeted metabolomics employing accurate mass spectrometry, were analysed and current analytical challenges critically evaluated. Discriminant and class modelling approaches (Support Vector Machine, Naïve Bayes, Decision Tree, Neural Network, partial least square discriminant analysis (PLS-DA), soft independent modelling of class analogy (SIMCA), and SIMCA-one class classifier) were used to determine which analytical technique or combination of techniques provides the best classification and prediction abilities. Good discrimination of carrots between different regions was achieved using metabolite and the ⁸⁷Sr/⁸⁶Sr isotopes/trace elements data, while data fusion on different levels (low, mid and high) showed promise with improvement of the classification results for some regions. It could be proven that the fusion of data from complementary techniques can provide more accurate knowledge about a sample origin and yield better inferences than a single technique.

Acknowledgements: The COMET-K1 competence centre FFoQSI GmbH.

Metrology in Food

Wednesday Late Morning Lectures

Apr 03, 11:30 - 12:15

OR-27

Calibration and measurement capabilities (CMCs) for food sample analysis

Radojko Jačimović, Tea Zuliani, Vesna Fajon, Polona Vreča, Milena Horvat

Jožef Stefan Institute, Department of Environmental Sciences, Slovenia

The Department of Environmental Sciences of the Jožef Stefan Institute (IJS/O-2) is Designated Institute (DI) for Amount of substance/Chemical trace Elements/in the organic and inorganic materials. Metrology Institute of the Republic of Slovenia (MIRS) gives us this status in year 2010. As a DI we can participate in key and supplementary comparison studies, which are traceable to the International system of units (SI). Successful participation in such studies can be used as a claim for supporting evidence of our Calibration and Measurement Capabilities (CMCs). The CMCs may be submitted only by national metrology institutes (NMIs) or national standard holders (NNEs) - Designated institutes (DIs) that are members of

one of the regional metrology organizations (e.g. for Europe is EURAMET) under the auspices of the CIPM MRA (Mutual Recognition Arrangement of national measurement standards and calibration and measurement certificates issued by national metrology institutes) and demonstrate international comparability of measurement capabilities - International equivalence of measurements that are traceable to the SI.

At the end of 2014, the IJS/O-2 applied for the CMCs entry to the Technical Committee on Metrology in Chemistry (TC-MC) within the framework of EURAMET in cycle XVI, which resulted from participation in key international comparisons (KCs), which were organized by Consultative Committee for Amount of Substance: Metrology and Chemistry and Biology (CCQM). The application contained a proposal for the entry of 4 CMCs in the field of chemical measurements in category 11 – Food according to the BIPM classification. Proposed 4 CMCs are as follows: As, Zn and Fe for Seafood and Total As for Brown rice flour. For these CMCs validated methods k0-INAA and ICP-MS were used. The proposed CMCs have been approved by technical committees of the EURAMET and BIPM and entered in the BIPM Key Comparison Database (KCDB) in July 2015.

OR-28

Key comparison studies on the ratio of the number of copies of specified intact sequence fragments extracted from different matrices

Mojca Milavec

National Institute of Biology, Department of Biotechnology and Systems Biology, Slovenia

Metrology, or the science of measurement, plays an important role in facilitating the comparability of results on an international basis. It investigates potential sources of variability between measurements and considers other factors influencing the measurement uncertainties. Bureau International des Poids et Mesures (BIPM) is the inter-governmental organization that has been established since 1875. Member states of BIPM act together on matters related to measurement science and measurement standards. This is achieved, amongst others, through the activities of the International Committee for Weights and Measures (CIPM), whose task is to promote worldwide uniformity in units of measurement. Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CCQM) is one of the bodies that advise the CIPM on matters related to different aspects of measurements and is responsible for developing, improving and documenting the equivalence of national standards (certified reference materials and reference methods) for chemical and biological measurements.

One of the working groups of the CCQM is dedicated to traceability and comparability of measurements of nucleic acids (Working Group on Nucleic Acid Analysis – NAWG). The measurement space of NAWG ranges from the chromosome, epigenome, genome, transcriptome, to regulome. The need to underpin higher order measurement capabilities is a central driver for the activities of NAWG. Members of NAWG are National Metrology In-

stitutes (NMIs) and Designated Institutes (DIs). Nucleic acid measurement approaches are prioritised which require support and demonstration of measurement capabilities, such as quantification of nucleic acids, nucleic acids sequencing and quantification of post-translational modification/epigenetics, while the sectors where these measurements are needed are stakeholder driven.

Some of the key sectors that require the development of traceable measurements and the demonstration of international equivalence of measurement results are food safety, food authenticity and nutrition. In order to demonstrate and document the measurement capabilities of NMIs and DIs, several studies have been organized by members of NAWG. The most relevant for the food sector is a series of Key Comparisons on quantification of genetically modified organism (GMO) content in different food matrices. The aims and the results of these studies will be presented.

OR-29

Quantification of measurement uncertainty using model equation: Case of determination of alpha-tocopherol content in plant oils

Erika Bešter, Bojan Butinar, Milena Bučar-Miklavčič

Science and research Centre Koper, Laboratory of the Institute for Oliveculture, Slovenia

Evaluation of measurement uncertainty for determination of alpha-tocopherol in plant oils is presented. Alpha-tocopherol is determined according to SIST EN ISO 9936: 2016 method. Sample is dissolved in heptane and analysed by high-performance liquid chromatography. Fluorescence detector is used for quantification. Alpha-tocopherol is quantified using five points calibration plot. Concentration of calibrant is determined by ultraviolet absorption. Measurement uncertainty was evaluated according to There are two major contributions to measurement uncertainty. The first contribution is random error which was calculated using withinlaboratory reproducibility data. The random error at within-laboratory reproducibility conditions was calculated using the data from control sample, analysed in two years period. The second contribution is bias which has further two contributions, bias of the method and laboratory bias. The bias of the method was evaluated using model equation, taking into account all steps from the method including determination of calibrant concentration, constructing the calibration plot, sample preparation, and sample analysis by HPLC; individual contributions to measurement uncertainty were evaluated and combined. Regular analysis of standard reference material (NIST SRM 3278) was used to estimate the bias of the laboratory; the uncertainty of the reference value was taken into account as well. The individual contributions to measurement uncertainty were as follows: 1.70% (laboratory bias), 2.44% (bias of the method) and 1.74% (random error). The calculated combined uncertainty was 2.72% and therefore the expanded combined uncertainty with coverage factor being equal to 2 and confidential limit 95% was 5.4%.

OR-30

Exploitation of crowdsourcing for collecting compositional data on prepacked foods and guiding healthy food choices with a smart-phone application

Urška Pivk Kupirovič¹, Anita Kušar¹, Barbara Koroušić Seljak², Igor Pravst¹

¹Nutrition Institute, Research group 'Nutrition and Public Health', Slovenia

²Jožef Stefan Institute, Computer Systems, Slovenia

Excess intake of calories, saturated fat, sugar, and salt derived from foods are a major cause of diet-related health issues in the population. Changes in the nutritional quality of the food supply are recognized as a key factor affecting public health. Monitoring and comparing the nutrient composition of prepacked foods between countries, food companies, and over time have a potential to drive changes in the nutrient composition of prepacked foods and support reformulation policies. Existing food composition database cannot keep up with the continuous reformulation, introductions and removals of packaged foods on the market.

Recently, a national project named "Innovative solutions for informed choices" was launched in Slovenia by the Nutrition Institute, the "Jožef Stefan" Institute and the Slovenian consumer organisation, and cofunded by the Ministry of health of Republic of Slovenia. Within the project, the existing "VešKajJeš" mobile app aimed to stimulate healthy food choices and food reformulation was upgraded, also to employ crowdsourcing to support compiling up-to-date database on the composition of prepacked foods. The idea is to enable easy comparison between different foods for the app users, and stimulate them to collect data of new foods, which are not yet included in the database. The users start the process with scanning barcode of the product, which is then used to match the food in the pre-existing database to interpret the nutrient composition on the phone screen. Food traffic light system based on total fat, saturated fats, total sugars and salt contents is used to support interpretation of the nutrient composition of foods.

Before launching the application, we collected and compiled data on the composition of over 22.000 prepacked foods available in the Slovenian food supply, enabling the app users to access data for the majority of the foods. For foods which cannot be found in the database, the app users are asked to take three photographs of the packaging (front of package, side with ingredient list, side with nutrition declaration), which are then transferred to our database. After evaluation newly send data of missing foods are added to the compositional database of prepacked foods. Crowdsourcing has a potential to significantly contribute to efficient monitoring of the food supply. Advantages of such an approach include improved costs, speed, quality, flexibility, scalability, and diversity. In the future, this

approach will be compared with costly, systematic data collection in food stores.

OR-31

Slovenian consumers' preferences towards traditional food products with national and EU quality labels

Mira Kos Skubic¹, Marija Klopčič², Mira Kos Skubic³, Anita Ule², Karmen Erjavec⁴

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Consumers are raising their awareness of and increasingly becoming more attentive to food traceability, origin and quality. On 14 December 2012, a new EU Regulation on quality schemes was published (Regulation 1151/2012/EU). European Commission developed a common policy facilitating the information flow between different stakeholders, including the consumers, farmers, food industry, etc., based on PDO - Protected Designation of Origin, PGI - Protected Geographical Indication, and other labels. EU quality labels should help consumers at informed decisions, as a control tool, with the label assuring the consumer of product traceability to the point of production, and that relevant know-how and skills had been applied. EU quality labels were adopted at national level, thus resolving the issue of asymmetric information. Several studies have shown a varied influence of EU quality labelling on consumer preferences between products and countries. Given the scarcity of studies on consumers' preferences for the EU and national geographical labels of different food categories in Slovenia, without tradition in EU quality labels, the aim of this study was to understand the Slovenian consumers' preferences towards cheese, ham and honey, labelled with the national and the EU PDO and PGI indications.

This paper presents the preferences of Slovenian consumers towards typical Slovenian food products with national labels, those with EU labels, and of different price segments or origin. An online survey with a representative sample of the Slovenian population of 650 consumers was conducted. Consumer preferences were estimated using choice-based conjoint analysis.

The study findings show that the price is most powerful in driving consumers' preferences for cheese and honey, and the origin for ham. The label is the least preferable attribute for all these products. However, cheese, ham and honey with the national PDO and PGI labels were more desired than their EU equivalents. The results show that around 65 % of respondents reported the awareness of two national Slovenian quality labels (national PDO and PGI), whilst the awareness of the EU PDO & PGI labels was reported only by 30.15 % and 36.62 % of respondents, respectively.

Traditional Slovenian food products have huge market potential in Slovenia and abroad, and capacity to create added-value products, and to interconnect local produc-

ers with the workforce in tourism.

**P O S T E R
P R E S E N T A T I O N S ,
A B S T R A C T S**

Lunch Break and Poster Session Apr 01, 12:45 - 14:00

PO-01

Geographical origin of plant food: Implementation of robust screening tests

Anja Mahne Opatič¹, Marijan Nečemer¹, Sonja Lojen¹, Rajko Vidrih²

¹Jožef Stefan Institute, Slovenia

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Recently, increased interest in production with declared and guaranteed geographical origin has resulted in higher market prices for these products, and thus mislabelling and mixing higher class products with cheaper products have become a serious problem in many areas of the food industry. The need to monitor the authenticity and quality of various types of plant samples led to increasing demand for rapid, inexpensive and reliable screening methods in order to check their origin. Therefore, our research work was focused on evaluation of potential traceability parameters for geographical characterization of different types of plant samples through the application of a broad spectrum of analytical techniques in combination with multivariate statistical analysis. Geographical characterization and classification of plant samples into groups was performed through applying different parameters such as stable isotopic compositions of four major bio-elements ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$, $\delta^{18}\text{O}$), elemental content (Na, Mg, P, S, Cl, K, Ca, Mn, Fe, Ni, Cu, Zn, Mo, Br, Rb, Sr) including rare earth elements (Sc, Y, Nb, La, Ce, Pr, Nd, Dy, Er), and physico-chemical parameters (total antioxidant potential, total phenolic compounds, ascorbic acid, lutein, nitrates and nitrites, ammonium), and data treatment using discriminant analysis. Subsequently, efficient preliminary statistical models were created. Classification efficiencies of suggested multivariate statistical models were found out to be sufficient for fast and robust screening purpose, giving the overall success rate of correctly reclassified samples for particular model from 71.1% to 100.0%. Different approaches were tested to assign geographical provenance at different scales- the macro-region origin at national scale (i.e. Slovenian) to organically grown garlic and potato, or country of origin at the larger European and Mediterranean scale to commercially distributed tomato, lettuce and sweet pepper. Present studies were performed to initiate the collection of data for the creation of fundamental and reliable traceability models since an important prerequisite for implementation of any official surveillance of origins is the availability of the relevant databanks, at both national and international levels. These protocols enable traceability and verification of provenance on selected realistic physico-chemical key parameters, and not only traceability through documentation, which is unfortunately the main common practice in our food trade market.

PO-02

On the traces of the *Cannabis sativa*

Marco Calvi¹, Barbara Stenni¹, Federica Camin², Luana Bontempo², Mario Baldini³, Nives Ogrinc⁴, Tea Zuliani⁴, Paolo Guarnaccia⁵, Clara Turetta⁶

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⁶Consiglio Nazionale delle Ricerche (Cnr), Istituto per la Dinamica dei Processi Ambientali IDPA, Italy

Hemp (*Cannabis sativa*) is becoming increasingly important year by year, especially in Italy, mainly in the food sector (oils, flours, seeds).

The aim of this PhD study is to define a method to trace and guarantee the geographic provenience of the Italian *Cannabis sativa* and its derived products. Nowadays, this method does not exist for the “industrial hemp”, except for Marijuana traffics (Elisa K. Shibuya et al., 2007, Janet M. Hurley et al., 2010).

This traceability study will be based on stable isotope ratios and on trace elements fingerprints. Food authentication has successfully been using this method, mainly through C, N, H, S and O stable isotope ratios, for a long time now.

First samples of hemp were collected in 2018 in Veneto, Emilia Romagna and Friuli Venezia Giulia regions. Further samplings are planned in 2019 and 2020, starting from early Summer (South Italy) to Autumn (North Italy). Two specific sites will be considered for the so called “Study of Hemp Factory”: Udine and Catania, that were chosen because of their difference in climatic conditions and geological background, helping us to understand which isotopic ratios will be more effective in the traceability along the whole production chain. Indeed, in the “Study of Hemp Factory”, we will evaluate oil, plant, inflorescences, seeds and fertilizers. $2\text{H}/1\text{H}$ and $18\text{O}/16\text{O}$ isotopic ratios of local precipitation and irrigation waters will be compared to the ones obtained from the plant tissues to understand if these ratios can be used as tracers. A further sampling campaign will be carried out in other areas of Italy for defining a spatial variability: here, only plants and oils will be analysed.

The isotopic composition of C, N, H, O and S, will be analysed by Isotope Ratio Mass Spectrometry at Fondazione Edmund Mach and the IDPA-CNR laboratories. H and O isotopic analysis on waters will be done at DAIS-Cà Foscari University.

Moreover, isotopic composition of Sr and Pb will be determined in plant, soil and oil samples at the Jožef Stefan Institute laboratories in Ljubljana, by means of Multi Collector Inductively Coupled Plasma – Mass Spectrometry. Simultaneously, to reinforce the method, ICP-MS analysis on trace elements will be performed on selected samples,

at IDPA-CNR Venice.

This multiparametric approach will allow us to define which parameters are more useful for trace back the geographic provenience of Cannabis sativa and its derived products.

PO-03

Application of chemometric tools for determination of authenticity of Serbian pear spirits

Biljana Marosanovic, *Maja Lojovic*, Aleksandra Bauer
SP Laboratorija, Serbia

In recent years, many food products have been losing their authenticity, uniqueness and characteristic of the area from which they originate. The authenticity of fruit spirits is one of the important issue regarding the consumer protection and it is based on the identification and elimination of fraud in the market. Pear spirit is a national, Serbian, alcoholic beverage with a great tradition and it becomes subject of falsification. Counterfeiting is related to ethanol content increasing, by adding sugar from sugar beet (C3 plant) or sugar cane and corn (C4 plant).

The determination of $\delta^{13}\text{C}$, $\delta^2\text{H}$ and $\delta^{18}\text{O}$ isotope ratios by Elemental Analyzer - Isotope Ratio Mass Spectrometry (EA-IRMS by Thermo) is a precise analytical method that can be used to check the authenticity, botanical and geographical origin of fruit spirits.

For detection of the adulteration, we have made laboratory control samples of spirits of pear, sugarcane and corn in SP Laboratorija. At the same time, we have prepared samples of pear spirits with different amount of added beet sugar (3%, 5%, 10%, 20% sugar was added on the weight of fruit during fermentation), from different geographical origin (South and North part of Serbia). Also, we collected pear spirits produced in Serbia in the period from 2005 until 2017. Combined results of $\delta^2\text{H}$ and $\delta^{13}\text{C}$ values gives an important information about botanical origin of ethanol and possibility of distinguishing between fruit spirits and spirits with non-fruit origin (made from beet sugar, maize, cane sugar). In the aim of geographical classification for spirit samples, originating from different locations in Serbia, spirits has been determined by isotope ratio $\delta^2\text{H}$ and $\delta^{18}\text{O}$.

Application of Chemometrics has established a linear correlation between the concentration of sugar values (derived from sugar beet) and $\delta^2\text{H}$ values. Applying this equation, it is possible to calculate the amount of added sugar in the unknown sample.

The application of the formed database enabled the classification of commercial pear spirits on the basis of botanical and geographical origin.

PO-04

Characterization of truffles (*Tuber* sp.) in Slovenia using stable isotope approach and elemental composition

*Bor Krajnc*¹, Marijan Nečemer², Federica Camin³, Katarina Vogel-Mikuš⁴, Staša Hamzić Gregorčič¹, Lidiya Strojnik¹, Nives Ogrinc¹

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⁴University of Ljubljana, Biotechnical faculty, Slovenia

The high cost of several species from the genus *Tuber* attracts more or less exquisite adulteration practices. The most known of the *Tuber* species in Europe are: *Tuber magnatum* Pico 1788 and *Tuber melanosporum* Vittadini 1831, followed by other commercially interesting species such as *Tuber aestivum* Chatin 1887, *Tuber macrosporum* Vittadini 1831, *Tuber brumale* Vittadini 1831, *Tuber borchii* Vittadini 1831, etc. Truffles are used in culinary as fresh fruiting bodies or as ingredients in the processed product. Since 2011 the truffle hunting is again legal in Slovenia and majority of Slovenian truffles comes from hunting wild truffles. While some of the species can be found in different parts of Slovenia, the two most appreciated *T. magnatum* and *T. melanosporum* are found only in Slovenian Istria.

In season 2017-2018, six truffle samples were collected in Slovenian Istria. Two samples of *T. magnatum*, one *T. borchii*, one *T. melanosporum*, and two *T. aestivum* were analyzed for elemental composition by X-ray fluorescence (XRF) and for stable isotope composition (H, O, C, N, and S) by isotope ratio mass spectrometry (IRMS). Stable isotope composition of carbon was also determined for the main volatile organic compounds by the use of headspace solid-phase microextraction (HS-SPME) coupled with IRMS.

There was high variability of $\delta^2\text{H}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ observed between different truffle species. The statistical evaluation of elemental and stable isotope compositions indicated that samples can be separated according to $\delta^{15}\text{N}$, Pb and P. $\delta^{13}\text{C}$ values in VOC were also significantly different between different truffle species. It was also speculated that different VOC components can be produced not only by the truffle bur also or exclusively by the microbes such as yeasts or bacteria.

These preliminary results of the ongoing study show that elemental composition and stable isotope composition could be used for determination of species variety and trophic status of truffles. It was also indicated that truffles offer a unique opportunity to better understand the ecological function of microbes associated with fungi and their involvement in aroma formation.

Acknowledgement: The research was performed within ARIMnet REALMed project with the aim of "Pursuing authenticity and valorization of Mediterranean traditional products".

PO-05

Establishing reproducibility in compound-specific stable isotope measurements for food authentication and adulteration

Chris Yarnes

University of California, Davis, Stable Isotope Facility, United States

Promising applications of compound-specific stable isotope analysis (CSIA) from carbohydrates, lipids, and proteins, as well as other compounds, have developed rapidly as new tools for food authentication and adulteration. However, as in the early days of bulk stable isotope analysis (BSIA), the establishment of best practices in quality assurance (QA) procedures has lagged behind the rush to applications. The lack of consistent QA procedures represents a significant hurdle to measurement reproducibility and the adoption of CSIA for routine food authentication.

Quality assurance in stable isotope analysis relies on four key principles for establishing reproducibility [1]. These include: (1) identical treatment, (2) identical corrections, (3) identical scaling, and (4) error detection. For BSIA, these principles are well-known and have been largely adopted by the analytical community. However, difficulties arise when adopting these principles within a CSIA context, partly due to the complexity and variety of analyses utilized by practitioners. This is particularly true with respect to the principles of identical scaling (3) and error detection (4). Many quality assurance procedures currently in use are insufficient to establish reproducibility.

Here I outline quality assurance procedures that meet the four principles described above, from four different analytes, each with different analytical features: 15N and 13C of amino acids of wild-caught fish, and 2H and 13C from fatty acids from olive oils. I demonstrate how variation in QA procedures impacts measurement accuracy, as well as the essential value of appropriate reference materials to assess the outcome quality of the applied QA procedures. These QA procedures allow for strict traceability and normalization to international reference standards through certified reference materials across a range of analytical contexts.

Standardized quality assurance and assessment practices will be important to establishing reproducibility in CSIA measurements, both within and among laboratories. It is both technically practical and desirable for CSIA practitioners to adopt quality assurance and assessment procedures similar to those outlined for BSIA to improve both measurement accuracy and traceability. In order to move from basic research to the broad-scale application of CSIA techniques within food authentication and adulteration, the establishment of expectations for quality control and analysis for CSIA will be required.

PO-06

Development of HILIC method for LC-MS analysis of glycolysis metabolites

Anže Pavlin¹, Martin Šala², Drago Kočar¹

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This research project is aimed at finding optimal method for separation of glycolysis metabolites using principles of Hydrophilic interaction liquid chromatography (HILIC). HILIC represents an effective separation

of small polar compounds on polar stationary phases.[1] The method for separation of 11 analytes was developed. That includes several glycolysis metabolites such as glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), dihydroxyacetone phosphate (DHAP), fructose-1,2-bisphosphate (FBP), glyceraldehyde-3-phosphate (GAP), 3-phosphoglycerate (PGA), phosphoenolpyruvate (PEP), pyruvate (Pyr), lactate (Lac) and cofactor nicotinamide adenine dinucleotide (NAD) and its phosphorylated form (NADP).[2] For the purpose different columns were compared. We tested Xbridge Amide 3,5 μ m; 3.0 x 100 mm (Waters), HILIC A 3 μ m; 100 x 3.0 mm (ACE), HILIC B 3 μ m; 100 x 3.0 mm (ACE) and HILIC N 3 μ m; 100 x 3.0 mm (ACE) exploring a wide range of parameters such as mobile phase constitution, pH, ion strength, gradient vs. isocratic. Ion strength of the mobile phase was tested in the range from 10 to 15 mM and the pH of buffer was set to 2.1, 3.0 or 4.7. The measurements were performed at room temperature. SRM transitions for all analytes were optimized using direct infusion, while the optimization of LC-MS method was performed on Perkin Elmer PE200 HPLC with mass spectrometer 3200QTrap (AB Sciex). Optimal results were obtained with Xbridge Amide 3,5 μ m; 3.0 x 100 mm (Waters) column where the concentration of buffer salt was 15 mM and the pH of buffer solution was 3.0. Using optimized experimental conditions, all analytes were resolved. Validation parameters of the method show that it can be used for determination of some metabolic processes, such as glucose uptake and lactate production as well as for monitoring of other glycolysis metabolites.[3]

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PO-07

Trust me? Consumer trust in expert information on food product labels

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Stable isotope analysis has become an important tool for tracing food origin and verifying its authenticity. When we aim to provide such scientifically based test results to consumers, “a label” on food product can be a feasible method. On the other hand, there are currently various types of product labels with various sources of label information. The research question of this study is: How do consumers evaluate label trustworthiness of expert labels based on stable isotope analysis in comparison to other commonly used label types?

We conducted a large-scale online survey in Japan, the USA, Germany, China and Thailand (n=2000 per country). For the purpose, we used professionally designed

labels for four food types (milk, honey, oil, wine) and 5 different sources of food information (farmers, government/administration, producer associations, experts, and consumers). First, we tested whether respondents could correctly identify the label information source to evaluate label legibility. We then asked respondents to evaluate the trustworthiness of labels with a literature-derived instrument that included six Likert-scale items ranging from overall label trust to purchase intent. Trustworthiness toward the sources of food information were also asked.

Results show that label legibility varied drastically among countries. The expert label had the lowest legibility. Nevertheless, respondents who correctly identified the information source of all labels chose expert labels as the most or second-most trustworthy across all countries and food types. In comparison, consumer labels received comparatively low levels of trust. Furthermore, trust in food information source is generally most, and moderately correlated with trust in the associated label. These results suggest that expert labels might play an important role as trusted sources of information in an increasingly complex global food system, although expert labels must be carefully designed to ensure they are legible and useful to consumers.

PO-08

Influence of the isotope signature of new fertilizers in the nitrogen isotope fingerprint of the organic foodstuffs

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The total area under organic farming in the EU is continuously increasing, covering more than 12 million hectares of agricultural land (2017). This type of agriculture is based on a reduction of synthetic phytosanitary and fertilizers and an increase of soil fertility incorporating carbon and nitrogen to the soil.

To increase soil fertility is a challenge in semi-arid regions, such as the south-east of Spain and numerous agricultural inputs are needed. In this sense, the organic farming supply industry is boosting their customers, their fertilizers formulations and their profits to this purpose. These enterprises transform waste into valuable agricultural inputs according to the new circular economy approach.

In spite of the severe control of organic farming in Europe, the European Parliament (2013/2091(INI)) approved a motion, where the organic foodstuffs are one of the most susceptible products of fraud. Moreover, it was stated that 'food fraud generally occurs where the potential for is high and the risk of getting caught are low.

To avoid this fraud, the isotopic signature of nitrogen ($\delta^{15}\text{N}$) of the organic products is an useful tool to verify that the consumers or any stakeholders in the organic farming food chain are trusted and the foodstuffs have been produced without synthetic fertilizers. This technique is based on the different isotopic signature of the organic and conventional fertilizers. Unfortunately, there is not an updated database of isotopic signatures of fertilizers. New fertilizers with different characteristics have ap-

peared during the last years and their consumption among farmers has increased a lot. Several examples are the que-lated microelements and formulated hydrolysed proteins and their isotopic fingerprints are still unknown.

In this regard, IFAPA is working on a project in which these new marketed fertilizers are monitored in order to avoid regulatory fraud. Our preliminary results showed that the major part of the chelated fertilizers had percentages of nitrogen ranging from 1 to 5% with $\delta^{15}\text{N}$ values lower than 0.76‰. Moreover, the formulated hydrolysed protein fertilizers showed percentages of nitrogen above 2% and a mean value of $\delta^{15}\text{N}$ of 2.21‰, including some of them with values of $\delta^{15}\text{N}$ lower than 0‰. Our results offered additional information about the characteristics of new fertilizers and the likely influence in the isotopic signature in the organic foodstuffs cultivated with them.

PO-09

Characterisation of white truffle using ICP-OES and IRMS

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White truffle (*Tuber magnatum* Pico) is a typical product of some Italian regions, and due to its organoleptic properties it is of high economic value. According to EU Regulation No 1308/2013, all agricultural products - including truffles - to be sold fresh to the consumer may only be marketed if their geographical origin is indicated. Most studies on truffles have considered in particular the composition of compounds constituting their aroma or have classified and distinguished the different truffle species genetically. Up to now, no analytical method is available to objectively determine the geographical origin of truffles. Stable isotope ratios of light elements in combination with the elemental composition have been used successfully over the last years to trace different food matrices. Therefore, the aim of this study was to verify if this type of approach could be useful also to trace white truffles.

In particular, an analysis of $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$, $^{18}\text{O}/^{16}\text{O}$, $2\text{H}/^{1}\text{H}$ was conducted in truffles using Isotopic Ratio Mass Spectrometry (IRMS). The elemental profile of Ag, Al, B, Ba, Ca, Cd, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Sr, Zn was carried out using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) after microwave digestion.

About twenty authentic white truffles from a number of Italian regions (Piedmont, Tuscany, Marche, Molise) were analysed. Despite the small number of samples, the first results seem to highlight the possibility to group according to origin, encouraging the extension of the survey to further samples.

PO-10

Food and beverage fraud prevention using isotope fingerprints

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Stable isotope measurements can differentiate between food and beverage samples which otherwise share identical chemical composition: this is called the isotope fingerprint. Using the isotope fingerprint of food and beverage products is a reliable technique for fraud detection offering conclusive answers on questions associated with origin, adulteration and correct labeling of food and beverage products. An overview of the interpretation of isotope fingerprints and the technology used is provided.

The food and beverage industry suffers from fraudulent activities that include incorrect labeling of products and adulteration, which has a significant impact on food and beverage safety, brand names and reputation and the market economy. Preventing food and beverage fraud is a key challenge that requires a reliable, cost-effective analytical process that can detect whether the labeled product is authentic or if it has been changed after the final manufacturing process, or alternatively if it has been independently produced, using alternative ingredients, but labeled as an original product.

Is your wine watered down? Are your vegetables grown using organic farming? Is the honey you bought naturally sweet? Did you purchase authentic Tequila? Explore how you can use carbon, nitrogen, sulfur, oxygen and hydrogen isotope fingerprints to trace unique answers to these and many more questions regarding origin and authenticity of your foods.

PO-11

Neutron Activation Analysis for the characterization and discrimination of processed animal proteins and insect meal

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Neutron activation analysis (NAA) is a very effective analytical technique for the determination of the elemental composition in a large variety of matrices. It is based on neutron irradiation of the samples therefore a source of neutrons such as a nuclear reactor is required. The neutron irradiation generates radioactive isotopes related to the constituents of the matrix. By using germanium detectors and certified standard materials the generated emissions spectra give information about the elemental composition. The technique provides a true bulk analysis then no sample dissolution is necessary and problems related with contamination or losses are therefore avoided. As different radioisotopes have different half-lives, counting can be delayed to allow interfering species to decay eliminating spectral interference. NAA allows minimum detection limits in the sub-ppm range providing an elemental fingerprint of the analyzed sample which can be used for a deep characterization, for provenance and discriminating studies. In this work NAA was used to study systematic differences in the elemental composition of different zootechnical meal,

including processed animal proteins (PAPs) of different species and insect meals. Chemometrics was exploited for variables' screening and data treatment. The scope of this work is to provide a very specific characterization of meal products that could be fraudulently add to compound feed in view of a species-specific recognition of unknown meals.

PO-12

An isotope analysis of the link between groundwater and milk in the Parmigiano-Reggiano cheese production area for food traceability

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Determination of the geographical origin of foodstuffs is becoming of increasing interest to consumers and producers, since it may be used as a criterion for certifying quality and authenticity. The Protected Designation of Origin (PDO) trademark has been assigned to numerous local products based on their area of origin. In order to obtain this designation, the raw materials must have been produced and processed in the specific region from which the product gets its name [1] [2]. For traceability study the stable isotopes composition of the elements such as O, H, C, N and S were monthly evaluated on groundwater, milk, hay and fodder samples from ten cattle-shed belonging to the "Consorzio del Parmigiano Reggiano" over a period of one year. The main aim of the investigation is the evaluation of the dependence of the isotopic composition of milk on food (hay and fodder) and water ingested by the cow and define the isotopic fractionation of water ingested/milk produced and milk produced/food supplied. The 18O/16O and 2H/1H isotope ratios of groundwater and local food are indicators of the area of the milk origin being affected by the climatic factors. From the first results obtained it was possible to notice a differentiation of the isotopic values of O and H between plain and mountain milk. There is a correlation between water and milk values belonging to the same cattle-shed. Even, the hay produced locally by breeders is different compared to the one purchased by import indicating that these parameters can increase the added value of producer's cheese. Overall, results of the present study could be helpful for consumers, the food industry and government regulatory agencies as it can prevent fraudulent labelling of organic food [3].

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PO-13

Phenolic profile of two autochthonous grape varieties from Serbia

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Grapes are considered as one of the richest sources of polyphenols among plants. These phytochemicals play an important role in grape characteristics, especially their quality. The phenolic composition varies among grape varieties. Autochthonous varieties are valued as typical representatives of the regions where they are grown, with the ability to provide wines with unique characteristics and distinction. This study was aimed to analyze similarities and differences in the polyphenolic profile of autochthonous and international grape varieties collected in a vineyard located in East Serbia. Research included two Serbian autochthonous varieties, 'Smederevka' (white variety) and 'Plovdina' (white variety with muted red color of the skin). All grape samples were in the stage of full maturity when analyzed. Different parts of berries, namely skin, seed, and pulp were studied for total phenolic content, total anthocyanin contents, and radical scavenging activity. The highest total phenolic content and radical scavenging activity were found in 'Plovdina' seeds, whilst among the skins, 'Smederevka' stood out. Using UHPLC coupled with Linear Trap Quadrupole (LTQ) and Orbitrap mass analyzer, a total of twenty derivatives of malvidin, delphinidin, petunidin, cyanidin, and peonidin were identified in red grape skins. According to semiquantitative data (ion intensities), malvidin 3-O-hexoside and peonidin 3-O-hexoside were the most abundant anthocyanins in the extracts of red grape skins. White variety, 'Plovdina' which is characterized with reddish skin, was also examined for anthocyanins, and it was shown to be abundant in hexosides of peonidin and delphinidin. Aside from the semiquantitative data on the anthocyanins, UHPLC-LTQ Orbitrap MS was used for the identification and quantification of non-anthocyanin phenolics, and specific profiles of autochthonous grapes were established.

PO-14

Assessment of differences among conventional and organic production of fruits based on phenolic profiles

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The aim of this study was to investigate the possibility of using phenolic profiles for authentication of organic production. Chemical traits of three blueberry cultivars ('Bluecrop', 'Duke' and 'Nui') and three strawberry cultivars ('Favette', 'Clery' and 'Alba'), which were grown in conventional and organic production were assessed. The production was in the village of Pambukovica, West Serbia, under the same agro-climatic and soil conditions. Results of spectrophotometric testing pointed to notable differences, as three blueberry cultivars had higher values of total polyphenol content (TPC), total anthocyanin content (TAC) and total antioxidant capacity (RSA) when produced organically. Strawberries 'Clery' and 'Alba' from organic production had higher values of TPC, TAC, and RSA when compared to the conventional production, while the opposite was found for the cultivar 'Favette'. Various phenolic acids and flavonoids (39 in total) were quantified by using liquid chromatography with mass spectrometry detection (LC-MS). Based on differences in phenolic profiles distinctions among blueberries and strawberries were assessed. Finally, changes in the contents of the specific polyphenols were evaluated by Principal Component Analysis (PCA) enabling deeper insight into the farming regimes.

PO-15

Improved detection of exogenous sugars addition to fruit juices by rapid determination of sugars $\delta^{13}\text{C}$ with HPLC-co-IRMS

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Stable carbon isotope ratio ($^{13}\text{C}/^{12}\text{C}$) of sugars in fruit juices are proposed as an important internal standard as detecting exogenous sugars addition. The conventional method for determining the $\delta^{13}\text{C}$ value of sugar (ENV 12140: 1997) involved purification, lyophilisation, and EA-IRMS analysis is time-consuming and prone to isotopic contamination and fractionation. This study improved the method for sugar $\delta^{13}\text{C}$ analysis by conducting a simple two-step SPE extraction and subsequent HPLC-co-IRMS analysis. The repeatability of total sugars $\delta^{13}\text{C}$ value ($r < 0.2\%$) of this method is better than that of conventional one. Moreover, determination of compound-specific carbon isotope of sugars by the HPLC-co-IRMS method enables us to establish criteria for juices authenticity by examining the intermolecular isotope correlations between sugars or between pulp and sugars. A linear regression model composed of analyses of 14 authentic orange juice from Taiwan showed a strong correlation between the $\delta^{13}\text{C}$ values for glucose and sucrose ($r^2 = 0.88$) and was sensitive to the HFCS addition with a detection limit $< 5\%$.

PO-16

Optimization of on-line bulk and compounds specific carbon isotope ratio measurements of foods and beverages by using flow injection/liquid chromatography isotope ratio mass spectrometry (FI/LC-IRMS)

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Rationale: On-line d13d analysis of LC-IRMS has been used for food integrity check such as honey and wines. However, it was not widely used on food and beverages authenticity applications in past decade. Generally believed that the analytical accuracy and operation of usual continuous system such as EA/GC-IRMS was better than LC-IRMS. Therefore, this study focused on optimizing the commercial modules improving both on bulk and compounds specific analysis (BSIA&CSIA), increasing the analytical accuracy and to extend the adulteration tests on different matrixes. **Methods:** EA-IRMS: d13C values of various high purity sugars were measured. Sucrose, maltose, glucose, fructose, trehalose dihydrate and raffinose pentahydrate were calibrated for laboratory reference materials (RMs) through IAEACH6/IAEACH7 calibration curve.; LC-IRMS: Two six-port valves individually from HPLC and LC-Isolink were connected directly by tubes for controlling the sample flow bypass (FI-IRMS mode) or through (LC-IRMS mode) the chromatography column. The manual injection loop(10ul) of LC-Isolink was substituted by the 100ul syringe of HPLC, so the carbon content range of injection was wider. The above two functions could be switched and adjusted by program or manual. The analytical program included four steps, calibration curve build, bulk sample analysis, quality control and compounds specific analysis, the first two steps run under FI-IRMS mode and others run under LC-IRMS mode. The calibration curve was built by RMs of sugars (-10.92~-27.15mUr). The mixed solution of RMs from glucose (-10.92mUr) and fructose (-11.25mUr) was used for quality control analysis. **Results:** Linearity test at low carbon content level (100 to 500 ng) showed ± 0.2 mUr variation. The quality control results of CSIA showed excellent accuracy results, glucose: -10.92 ± 0.09 mUr; fructose: -11.31 ± 0.08 mUr (n=69). A linear regression model composed of BSIA results of honey between EA-IRMS and FI-IRMS showed a strong correlation, $R^2 = 0.9968$ (n=1567). **Conclusions:** The analytical protocol and instrument setting in this study simplify the on-line stable carbon isotope analysis of food and beverage and improve the uncertainty of measurement. The FI-IRMS system has be proved that could replace the EA-IRMS methods of bulk d13C analysis. In the follow-up study, the d13C analysis method has be successful built for individual sugars/organic acids in fruit juices through FI/LC-IRMS.

PO-17

Determination of geographical origin of different fruit and vegetables using stable isotope ratio mass spectrometry and element analysis

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The aim of this papet is to present the results obtained in the framework of the preliminary expert task of checking the origin of selected fruits (strawberries, cherries, apples) and vegetables (garlic) using stable isotopes of light elements and elemental composition in combination with multivariate statistical methods.

The samples were obtained from the regional units of the Administration of the Republic of Slovenia for Food Safety, Veterinary and Plant Protection, from the different geographical areas of Slovenia, where the selected samples of fruit and vegetables are produced. The survey includes a total of 123 authentic samples and 28 test samples from the market, among others, from abroad (garlic from Egypt, Croatia, China and Spain; apples from Serbia, New Zealand, Poland and Italy).

The elemental content of each sample (K, Ca, S, P, Mn, Cl, Br, Sr, Zn, and Rb) was determined by X-ray fluorescence spectrometry (XRF) and the isotopic composition of oxygen in water and carbon, nitrogen and sulphur in fruit pulp and vegetable using an isotope ratio mass spectrometer. The results were statistically evaluated using multivariate statistical methods, including principal component analysis (PCA) and discriminant analysis (DA). Test samples of strawberries and cherries showed statistical similarities with genuine samples, which suggest that samples are probably of Slovenian origin. Further, we managed to separate genuine Slovenian garlic samples from the foreign samples obtained from the market. The main parameters that influence this separation are concentrations of Cl, Sr, and $\delta^{18}\text{O}$ and $\delta^{34}\text{S}$ values. In the case of apple samples, good separation was obtained between authentic and test samples from abroad. Samples of apple that were declared as Slovenian overlap with genuine samples and confirm the correct declaration.

Discrimination analysis was used to separate samples of apples and garlic according to their geographical region and good separation between the Pannonian, Mediterranean, Dinaric and Alpine regions was obtained. The most discriminating parameters were $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values and the levels of S, P, K and Sr for garlic and $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values and the levels of Ca, K, Cl in apples. Overall prediction ability was 100% for garlic and 78.4% for apples.

The models built using authentic fruit and vegetable samples in the framework of this preliminary study provide a good basis for discriminating between authentic samples and those on the market with a certain degree of reliability. This study also underlines the need for further research.

Characterization of Spirulina food supplements from Slovenian market through element composition and stable isotope ratio of light elements

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Spirulina food supplement is a source of various nutrients and a good alternative food source that contributes to human health [1]. Environmental conditions such as climate change and variability and location of production that influence algal growth may affect potential biomarkers [2]–[3], but also the stable isotopic composition of carbon, nitrogen and sulfur. These parameters could be used to discriminate between the origin of microalgae and the adulteration of algal products. To our knowledge no research has been performed in the field of stable isotope ratio and geographical origin determination on Spirulina dietary supplements.

Forty-seven Spirulina samples were gathered from the Slovenian market for this research. The samples were in tablet, capsule, powder or fresh form, originating mainly from Hawaii, China, India, Taiwan. In this study macro- and microelement content was analysed in Spirulina using X-ray fluorescence (XRF) technique and trace element content using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Stable isotope ratios of hydrogen, carbon, oxygen, nitrogen and sulphur were analysed by Elemental Analysis - Isotope Ratio Mass Spectrometry (EA-IRMS) and high temperature TC/EA-IRMS to determine quality of the products on the Slovenian market through determining their authenticity and geographical origin.

Distribution of the elements following their concentrations in the samples was as follows: potassium > phosphorus > sulphur > silicon > calcium > chloride > iron > manganese > titanium > zinc > bromide > vanadium > selenium. No upper limits were exceeded for toxic trace elements such as arsenic, mercury, cadmium and lead. Nitrogen stable isotope values (¹⁵N) ranged between -5,4‰ and 13,8‰, ¹³C ranged between -32,3‰ and -16,7‰ and ³⁴S values ranged between -1,8‰ and 13,8‰. Discriminant Analysis (DA) of analysed elements and stable isotopes showed significant differentiation of samples originating from Italy, Taiwan and Hawaii. Separation of samples originating from other countries was less evident. Statistical analysis was done to differentiate between the samples of different form but the same producer and declared composition. Results show significant separation of samples according to their form.

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PO-19

Characterisation of bottled waters by stable isotope and multi-elemental analysis

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Bottled water is assumed by consumers to be a reliable and of high quality food product, because it is supposed that originate from remote and unpolluted areas. As the consumption of bottled waters is increasingly growing and thus, due to its ready availability, the potential of fraud is significant. The property of water depends mostly on the characteristics of the recharge area of the wells from where the water for bottling is extracted. As waters originate from various parts from the hydrological cycle and have distinct geological backgrounds, their natural origin is reflected in their hydrogen ($\delta^2\text{H}$), oxygen ($\delta^{18}\text{O}$), dissolved inorganic carbon ($\delta^{13}\text{CDIC}$) and strontium ($^{87}\text{Sr}/^{86}\text{Sr}$) isotopic compositions.

In the present study isotopic composition of H, O, CDIC and Sr together with the multi-elemental composition data were used to characterise 8 bottled (still) waters originating from Slovenia. Additionally, bottled waters from Austria (1), Croatia (1), Serbia (1) and France (2) were analysed in order to estimate the variability in composition.

The concentrations of trace elements in all water samples were low. They were lower than the threshold levels set by the Slovenian and EU directives on bottled waters, and US EPA and WHO recommendations. The $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio for Slovene waters ranged from 0.70795 to 0.71858, while other waters ranged from 0.70403 (water originating from volcanic area) to 0.70901. The isotopic composition of hydrogen and oxygen varied from -77.9 to -56.6 ‰ and from -10.84 to -8.32 ‰, respectively. The values can be interpreted as typical of groundwater from shallow aquifers reflecting the influence of infiltration of recent precipitation as the main water source. Inorganic carbon in natural water consists of gaseous and dissolved CO₂, dissolved carbon acid, dissolved hydrogen carbonate and solid calcium carbonate. $\delta^{13}\text{CDIC}$ depends on the contribution of these multiple sources of carbon. $\delta^{13}\text{CDIC}$ in our study ranged from -17.3 to -6.2 ‰ indicating different sources of CO₂ in bottled waters. Bottled water with the lowest $\delta^{13}\text{CDIC}$ value (-17.3 ‰) is characteristic for volcanoclastic environments and more soil CO₂ contribution, while with the highest $\delta^{13}\text{CDIC}$ value (-6.2 ‰) for carbonate environments with less soil CO₂ contribution.

The results of the investigation will be used to elaborate a spatial prediction map that will be further used for verification of geographical origin of bottled waters on the Slovenian market.

Nuclear magnetic resonance in food safety and quality

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For the food industry and consumers, the authenticity of foods is important. This has been highlighted by recent food scandals like the distribution of questionable meat in Poland. Traditional methods of analysis have limitations: either compounds of interest need to be known beforehand or sample preparation is expensive and time-consuming. Therefore, the food industry needs rapid methods of non-specific analysis like NMR.

NMR is a spectroscopic technique used for measuring the amount of radiofrequency radiation absorbed by atomic nuclei with magnetic properties. Number, shape, and intensity of a specific signal give us information about the magnetic environment of a certain type of nuclei and can be used for identification and structural determination of molecules. For samples with spectra that obtain good signal separation, a quantitative determination of specific molecule content can be done by editing the known amount of a standard substance. NMR spectroscopy can be applied in food safety and quality, having the advantage over other techniques as it has intact and time-saving sample preparation.

Proton spectra can reveal characteristic peaks for additives in food samples, like sucrose in wine. As hydrogen is the most abundant atom in organic molecules, ^1H spectra can get crowded, and less abundant metabolites get hidden beneath signals belonging to more concentrated metabolites. Therefore, other nuclei with non-zero spin properties are used in NMR analysis like ^{13}C , ^{15}N , ^{19}F and ^{31}P isotopes. Another approach to overcome spectra crowding includes 2D NMR experiments such as COSY, TOCSY, HSQC, and HMBC. To achieve exposure of signals belonging to less abundant metabolites, certain pulse sequence that saturate more concentrated metabolites can be used. A promising approach in food control is spectral fingerprinting. Spectra of many samples of the same food with different features are recorded to establish a database, which is used for further classification of samples. Different features can be geographical origin, organic/conventional farming, authenticity control, etc. Precise statistical methods are required to obtain meaningful results.

NMR spectroscopy is useful for determining indicators of food quality, such as unsaturated fatty acids in oils, for identification of additives, food origins, the year of production, etc. The main advantage of NMR is that the samples remain intact and can be efficiently prepared.

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Potential of stable carbon isotope analysis for compliance checking of corn mixed bread

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Slovenian market offers an extensive selection of breads, produced from wheat flour and mixtures with other flours. Corn mixed bread must contain at least 30% of corn flour in the total quantity of flour. Its sensory properties must be characteristic of this product type (Rules on the quality of bakery products, 2015). It has pale yellow colour, however very bright coloured corn breads are present in the market, putting under question the sufficiency of the corn flour content.

The carbon isotope composition of corn differs considerably from other cereals. The objective of the work was to investigate the potential of stable carbon isotope analysis for compliance checking of corn mixed bread. Seven laboratory bread samples were prepared, with 0-50%, and 100%, respectively, corn flour. The seven corn-wheat flour mixtures used for bread preparation represented the subgroup of samples. Six corn bread products and four corn flour samples were taken from the market. The $^{13}\text{C}/^{12}\text{C}$ isotope ratios expressed as $\delta^{13}\text{C}$ values were determined by Elemental Analyser coupled to Isotope Ratio Mass Spectrometry. A panel of 30 consumers assessed the appearance, aroma and liking of the laboratory bread samples using the ranking method and a 9-point hedonic scale, respectively.

The $\delta^{13}\text{C}$ values in model breads and corn-wheat flour mixtures were in strong correlation ($r=0.9904$; $p<0.001$). Equation of linear regression describing the relation between percentage of corn flour in bread and $\delta^{13}\text{C}$ values was determined. Percentages of corn flour in the commercial bread samples estimated by the equation ranged from 18.2 to 28.5%. In the sensory analysis, consumers selected the model bread samples with 50%, 40% and 100% corn flour to have the most typical appearance and samples with 50% and 40% corn flour the most typical aroma of corn bread. Samples with 30% and 10% corn flour achieved the highest hedonic scores.

Despite strong correlation between $\delta^{13}\text{C}$ values of flour mixtures and model breads with the same corn flour percentage, the results of commercial bread showed the need for more extensive research on $\delta^{13}\text{C}$ values in this bread, since variable contents of bread ingredients may affect these values. Variability in $\delta^{13}\text{C}$ values of commercial bread support the hypothesis that the corn flour percentage might be lower than legally set minimum. Hedonic scores suggest that consumers are more accustomed to lower contents of cornflour in this bread, which reflects the market situation.

How to establish stable isotope databases for authenticity assessment of aromas

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The demand for flavourings is increasing and today's consumers, more than ever, demand naturally flavoured products. Because flavour compounds are commonly falsified by either dilution, mixing with synthetic compounds, or false declaration of origin of natural resources there is a demand to control authenticity. At this point, gas chromatography isotope ratio mass spectrometry (GC-IRMS) is the most suitable method capable of distinguishing between natural and synthetic aromas. To verify the authenticity of commercial apple and strawberry distillates, $\delta^{13}\text{C}$ values of different aroma compounds must be determined and confirmed by comparison to $\delta^{13}\text{C}$ values of authentic compounds held in stable isotope databases. Any sample with one or more compounds outside this range is then suspected of being adulterated. Consequently, it is important to develop databases containing flavour compounds with well-defined origins. In this study, we developed stable isotope databases for apple and strawberry aroma compounds. First, we characterised 18 apple and 9 strawberry laboratory produced recovery aroma samples and 32 pure synthetically derived aroma compounds. We then used the results to establish a database of $\delta^{13}\text{C}$ values of 17 apple aroma compounds and 27 strawberry aroma compounds with regards to their origin (synthetic and natural). In the second step, we expanded these two databases by adding more authentic natural samples. Because sampling aroma volatiles, recovered in the water phase after steam distillation, is a timely and costly process, we also tested the fruits directly. The results show that raw samples or juices can be used for building a database, with the limitation that the number of aroma compounds that can be analysed is lower. By testing other fruits (peach, blueberry, watermelon, pear, banana, raspberry) we also determined if the type of fruit is an important parameter when creating a database. The results revealed differences in certain aroma compounds in different types of fruits. For most of the selected aroma compounds, there is good discrimination between the range of values for natural and synthetic authentic aromas, but despite this, certain aroma compounds had overlapping $\delta^{13}\text{C}$ values, meaning it was not possible to discriminate between natural and synthetic aromas with a high degree of confidence. An accurate determination of authenticity is, however, feasible using a multi-analysis approach such as GC-C/P-IRMS ($\delta^{13}\text{C}$ and $\delta^2\text{H}$ measurements).

Using stable isotope databases for authenticity assessment of commercial flavoured products

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Flavour is an important quality trait of food and beverage, and is determined by taste and aroma. However, the growing demand for natural aromas and that natural raw materials are becoming more expensive, which is putting increasing pressure on prices and quality. Moreover, owing to the price advantage that synthetic aromas have over natural ones together with the difficulty in differentiating between natural flavours from their synthetic analogues, means that synthetic flavours are being passed off as being natural. Apart from fraud, the addition of non-authentic compounds could pose a potential health risk. Consumer confidence can also be put at risk by passing off an inferior product as the genuine item. When assessing authenticity, the two most valuable assets are (1) a suitable analytical technique that can distinguish between the natural and synthetic compounds and (2) a database of authentic natural and synthetic aroma compounds of the compounds most important in defining the aroma. In regards to the former, gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) with headspace solid phase microextraction (HS-SPME) is an appropriate tool for assessing the authenticity of fruit aromas. For example, HS-SPME GC-IRMS analysis of $\delta^2\text{H}$ and $\delta^{13}\text{C}$ can be used to distinguish between natural and synthetic vanillin. In regards to the latter, we have used the database of authentic samples of apple and strawberry aroma compounds constructed within our laboratory to assess authenticity of natural flavouring products: natural commercial distillates, pure aroma compounds and powder flavour supplements, apple flavoured water and commercial food products flavoured with natural vanillin (yoghurt, ice cream, pudding and tea) were all tested. The authenticity of each was assessed by comparing $\delta^{13}\text{C}$ values, and in case of vanillin also $\delta^2\text{H}$ values (determined using GC-P-IRMS), with the isotopic values of authentic samples. The results of commercial samples show possible falsification for several fruit aroma compounds and that all the samples reported as being flavoured with natural vanillin contained synthetic vanillin. As these results indicate, significant doubt exists about the authenticity of flavoured products on the market and extensive testing of products is necessary.

Parameters for discrimination between organic and conventional production: a case study for chicory (*Cichorium intybus* L.)

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Chicory (*Cichorium intybus* L.) is an appreciated leafy vegetable among producers and consumers, especially due to its undemanding cultivation and the content of several bioactive substances. Many vegetable producers over the world switched to the organic production and the control of frauds became increasingly important over past decade. Six different fertilization treatments (a control – CONT, two organic – ORG1 and ORG2, two mineral – MIN1 and MIN2, and a combination of organic and mineral fertilizer – ORG1 + MIN1) were applied during growth of five chicory cultivars. The following cultivars were studied: three red coloured (Treviso, Verona, Anivip), one red-spotted (Castelfranco), and one green (Monivip). For each of the five cultivars, the same six fertility managements were applied in a completely randomized factorial design in plastic planting pots glasshouse experiment. The leaves were collected after four months growth and prepared for further analyses in two ways. Fresh leaf tissue was homogenized using the Ultraturax homogenizer or lyophilized and then ground to a fine powder using the ball mill. The analyses of dry matter, total phenolic compounds (TPC), total flavonoids (TFC), antioxidant potential (AOP), nitrogen assimilation (NH₄-N, NO₂-N + NO₃-N), stable isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$) and multi-element composition (P, S, Cl, K, Ca, Mn, Zn, Br, Rb, Sr, Mo) were performed in term to predict the organic or conventional production. The highest $\delta^{15}\text{N}$ values were determined when organic fertilizers were applied, while the lowest $\delta^{34}\text{S}$ values were found in case of fertilization with mineral fertilizers. Organic fertilizers increase the content of elements P and K, while mineral fertilizers increase the content of S. The combination of organic and mineral fertilizers resulted in higher AOP, TPC and TFC. Regarding nitrates, no significant differences were found between organic and mineral fertilization, although chicory plants grown in the control treatment contained significantly less nitrates. Data of analyzed twenty parameters for a total of 30 chicory samples grown up with six fertilization treatments (CONT, MIN1, MIN2, ORG1, ORG2, ORG1+MIN1) were evaluated using LDA. All six fertilization treatment groups were well separated between each other and 91.6 % of the original grouped cases were correctly classified. Function 1 explained 71.0% and function 2 20.6% of the total variance. Major contributors to discriminate in function 1 between different fertilization treatments were Br, Rb, K, Mo, dry matter and NO₂-N+NO₃-N, meanwhile Ca, Sr, TPC, AOP, $\delta^{34}\text{S}$ and Cl in function 2.

PO-25

Evaluation of carbon and nitrogen isotope analysis for authentication of organic versus conventional pork products

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A robust scientific technique, or combination of techniques, for the authentication of foods labelled organic would be of immense value for consumers, government agencies and the agri-food industry and stable isotope analysis has emerged as a promising tool for this purpose (Bahar et al., 2008). The carbon and nitrogen isotopic compositions of animal tissues can be a potential tool to identify animal diets (proportion of C3- and C4- plants), geographical origin or animal husbandry practices and hence are useful for authenticating products from the organic rearing systems. However, seasonal variations in the isotopic composition was detected in organic and conventional Irish beef, probably reflecting seasonality in animal feeding practices modulated by tissue turnover rates (Bahar et al., 2008). Most of all, the mechanisms of isotopic fractionation between an animal diet and its tissues are still not well understood (Bielefeld Nardoto et al., 2006) and they could be dependent on breed types. Therefore, such seasonal and breed variation needs to be considered in the isotopic authentication of any animal-derived products. On the other hand, discrimination among Iberian pigs feeding regimes has been achieved by the $\delta^{13}\text{C}$ of subcutaneous fat and corresponding fatty acids methyl esters (FAMES) (Recio et al., 2013; Delgado-Chavero et al., 2013).

The purpose of AUTENFOOD project (COM15-1-0035) is to evaluate the potential of these isotopic tools to distinguish meat and processed products from pigs reared in an organic production system compared to those reared in a conventional one. The motivation of this research comes from the need to protect this product of high quality and cost, from possible commercial fraud. To this goal, firstly variability of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of diverse diet samples, corresponding to different stages of organic and conventional pigs' feeding, has been tested in order to check if they were significantly different. Secondly, determining whether these potential differences are reflected in the bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of muscle, fat and hair as well as in the $\delta^{13}\text{C}$ of FAMES for each group of pigs taking into account different breeds (50% Pietrain, 25% Landrace, 25% Large White, 85% Duroc 15% Landrace and 75% Duroc 25% Landrace) and growing seasons along a year. These results will be compared to a similar recent study in China (Zhao et al., 2015). In order to, ultimately, know if different organic pork processed products could be discriminated in the market by this approach.

PO-26

Can isotopic fingerprinting distinguish unequivocally among Spanish beers?

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Beverages are among the most likely foods to be the target of food fraud (Kamiloglu, 2019) and although beer has the largest share of the global alcoholic beverage market, only limited studies of beer have been performed in terms of isotopic compositions (Bong et al., 2016). As the extent to which sugars or starches besides those

from malted barley are used is unknown for most beers (producers are not required to list ingredients), the bulk carbon isotopic composition ($\delta^{13}\text{C}$) has been used to determine the amount of C3- (barley, rice, wheat) and C4- (corn, sorghum, sugar cane) plant sources used in brewing (Brooks et al., 2002). However, industrial CO_2 addition may take place in order to strengthen poorly carbonated beers, so the combination of $\delta^{13}\text{C}$ of CO_2 and ethanol improved authenticity and distinction of beers (Calderone et al., 2007). Moreover, $\delta^2\text{H}/\delta^{18}\text{O}$ composition of beers can determine their geographical origin because of a strong correlation, consistent with natural waters but offset (probably due to the brewing process) from the Global Meteoric Water Line (Carter et al., 2015).

Among the different methods of distribution of beer, the kegs have acquired an important sales growth in Spain, but fraudulent re-filings are plausible. The aim of this study was to evaluate the potential of the isotopic fingerprinting to distinguish unequivocally a commercial brand of beer among other Spanish beers in the market for its traceability in case of a keg manipulation or health issue. For this purpose, first of all, it has been validated the reproducibility of the carbon isotopic composition of the ingredients (malted barley, adjuncts and hops) and the $\delta^2\text{H}/\delta^{18}\text{O}$ of the water used for its production in three different factories located in Spain quarterly during 18 months. The same was done for three types of beers (one of them without alcohol) produced in these three factories. They were all characterised in terms of $\delta^{13}\text{C}$ of CO_2 , ethanol or sugars (glucose and maltose) measured by HPLC-IRMS and $\delta^2\text{H}/\delta^{18}\text{O}$ of the product and compared with different lots of 6 other Spanish commercial brands to evaluate if they could be distinguished.

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PO-27

Arsenic compounds in rice and rice drinks available on the Slovene market

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Rice (*Oryza sativa*), wheat and maize directly supply more than 42 % of all calories consumed by the entire human population. Of them, rice is by far the most important food crop for people in low-income countries. In the developed world, rice consumption is lower but people who suffer from various allergies and some vegans/vegetarians often replace wheat with rice, and cow's milk with plant-based alternatives like rice drinks.

Arsenic-contaminated soil and irrigation water in major rice producing areas present a source of arsenic taken up by rice. Subsequent arsenic translocation, transformation and accumulation in the rice plant depend on the chemical form of arsenic (speciation), the growing conditions and the rice genotype. Grain-arsenic accumulation poses considerable health risks to humans in case inorganic arsenic (AsIII and AsV) exceeds certain concentration levels. The EU specifies maximum allowable inorganic arsenic levels of 200 $\mu\text{g}/\text{kg}$ (white rice) and 250 $\mu\text{g}/\text{kg}$ (parboiled and brown rice); organoarsenic species such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are in general less toxic. This study aims to investigate several types of rice and rice drinks available in Slovenia for their total arsenic content and more specifically their arsenic species.

Twenty four rice samples from Slovene shops were subjected to i) total arsenic analysis by microwave digestion (HNO_3) followed by ICP - MS, and ii) arsenic speciation by an extraction step with 1 % HNO_3 (efficiency, 85.9 ± 13.4 %) followed by HPLC-HGAAS. Total arsenic concentrations ranged from 56.9 to 258 $\mu\text{g}/\text{kg}$ (dry rice), with on average 74.6 ± 15.7 % of As present in inorganic form (mainly AsIII), and 20.5 ± 12.5 % present in the form of DMA. Twelve rice drinks contained from 4.1 to 27.7 $\mu\text{g}/\text{L}$ of arsenic; inorganic arsenic in the liquid part of all rice drinks ($1.7 - 22.8$ $\mu\text{g}/\text{L}$) was the main arsenic form. Like in the case of rice, some DMA ($0.1 - 4.5$ $\mu\text{g}/\text{L}$) was also present; MMA was only detected in low amounts in a few rice and rice drink samples.

We can conclude that none of the rice or rice drink samples investigated in our study exceeded the maximum allowable inorganic arsenic limits set by the European Union, although some samples were very close to the limit, thus justifying regular monitoring of foodstuffs available on the Slovene market.

PO-28

Exposure to mercury during prenatal period

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Seafood is major source of exposure to organic mercury (mostly as monomethyl Hg; CH_3Hg^+ , MeHg) for general population. The central nervous system is the primary target site where toxic effects of MeHg are manifested. Environmental disasters showed that acute prenatal exposure to high doses of MeHg result in different irreversible dysfunctions and anomalies. On the other hand, effects of chronic prenatal exposure to low-levels of MeHg on child development remain controversial and conclusions inconsistent.

The aim of this study was to measure blood MeHg concentrations in a population chronically exposed to low-to-moderate levels of Hg through seafood consumption and/or amalgam fillings and to evaluate if the Hg specia-

tion provide additional information on exposure and effect assessment.

Mothers from coastal Croatia (n=234) and central Slovenia (n=571) and their newborns (n=210, N=435, respectively), participants of EU project PHIME, were included in the study. We used existed data set of personal and lifestyle characteristics, THg in maternal and cord blood, and Apoe genotypes of mothers and children. We analysed MeHg in maternal blood (n=225) and in cord blood (n=436) with CVAFS (Tekran 2700, Canada). Child neurodevelopment was assessed at 18 months of age using Bayley-III. Statistics: STATA.

Geometrical means of THg in maternal and in cord blood were 2.05 ng/g and 1.90 ng/g, respectively. MeHg concentrations in maternal and in cord blood were 1.50 ng/g and 1.58 ng/g, respectively. In average, MeHg presented 70 % of THg in maternal blood and 86 % in cord blood with considerable range of 4-100 % for maternal and 8-100 % for cord blood. We observed that with lower MeHg concentrations the average percentage of MeHg decreases. Correlations and multiple linear regression confirmed the major source of exposure to Hg in our population was seafood consumption, presenting exposure mainly to MeHg. Concentrations of MeHg in maternal blood were associated also with pre-pregnancy body mass index, maternal age and plasma selenium levels. With direct measurements of MeHg in cord blood we showed that multiple linear regression models estimating associations between child's cognitive score and cord blood MeHg (adjusted for child's and/or maternal Apoe genotype) enabled better prediction in comparison with the models estimating child's cognitive score and cord blood THg (also adjusted for child's and/or maternal Apoe genotype).

PO-29

Optimization ultrasound-assisted method for extraction bioactive compounds from garlic (*Allium sativum*) sample using response surface methodology

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The definition of an antioxidant is a bioactive compound which can inhibit or delay the oxidation of other molecules. Antioxidants are categorized into natural and synthetic antioxidants [1]. Commonly used synthetic antioxidants include butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propylgallate and tertbutylhydroquinone. Reports of negative effects of synthetic chemical preservatives on human health has led to a desire to replace these synthetic chemical preservatives with natural preservatives showing antioxidant and/or antimicrobial activities [2].

Garlic (*Allium sativum* L.) has a reputation as a therapeutic agent for many different diseases such as microbial infections, hypertension, hypercholesterolaemia, diabetes, atherosclerosis and cancer. Health benefits of garlic depend on its content of biologically-active compounds, which differs between cultivars and geographical regions. Bioactive compounds, such as polyphenols, are of great

interest due to their health benefitting antioxidant properties and possible protection against inflammation, cardiovascular diseases and certain types of cancer.

The aim of our work is developed ultrasound-assisted extraction (UAE) method for extraction of total phenolics from garlic sample. Maximum retention of polyphenolic compounds during extraction requires optimised process parameter conditions. The influence of extraction parameters including time (min, X1), extraction temperature (oC, X2), methanol concentration (% v/v, X3), and solvent-to-solid ratio (mL/g, X4) on the extraction of total phenolic (TPC) and total flavonoid content (TFC) was modeled by using a quadratic regression equation. The optimal UAE conditions were 10 min extraction time, 80 oC extraction temperature, 65 % methanol concentration and 20 mL/g solvent-to-solid ratio. Methanol concentration and liquid-to-solid ratio were the significant parameters for the extraction process ($p < 0.01$). Under the UAE optimised conditions, the recovery of TPC was 1.324 mg GAE/ g FW (mg gallic acid equivalent/g fresh weight, approximating the predicted content (1.356 mg GAE/ g FW). When bioactive compounds extracted from garlic using UAE compared with Soxhlet extraction, it was also observed that total flavonoid content (28.97 ± 0.05 mg RUE/g FW) were higher in the UAE extracts. These findings further illustrate that extraction of bioactive compounds from plant materials using UAE method consumes less extraction solvent and saves time.

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PO-30

Comparison of new developed non-esterification with standard method for determination fatty acids in milk sample

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Oil and fats in food are composed of different types of fatty acids (FA): monounsaturated, polyunsaturated and saturated. Naturally, all unsaturated FAs are in cis form, whereas a large portion of unsaturated FAs isomerize to their trans form counterparts during industrial hydrogenation. Accurate quantification of FAs in dairy products is important for quality control, nutritional, antimicrobial, authenticity, legislative, and flavor purposes. For these reasons, we have developed and validated a new method and compared it with the standard method for determination fatty acids in milk sample.

The critical process in GC analysis of fatty acids is the required methylation of the fatty acids to obtain fatty acid methyl esters (FAMES). Many different methylation methods are described in the literature but the most commonly used are those catalyzed by an acid, base or boron trifluoride and methylation with diazomethane, each of which have advantages and disadvantages and differ in

their applicable range.

The advantage of acid-catalyzed and boron trifluoride methylation, in which H₂SO₄ and BF₃ are widely used, is that free fatty acids (FFAs) can be catalyzed for esterification. However, higher temperatures, usually between 60 and 90 °C, and longer reaction times, which could affect the compositions of fatty acids, can lead to not only inaccurate results but also the occurrence of side reactions, loss of the unsaturated FAMES and isomerization. On the other hand, the advantage of non esterification method are mild extraction conditions and fast procedure for sample preparation.

The proposed GC method without esterification was validated, exhibited good accuracy and precision, and was successfully applied to the quantification FAs in milk. A comprehensive validation was undertaken to establish method linearity, limits of detection, limits of quantification, accuracy, and precision. Linear calibrations of 20 to 700 mg/L ($R^2 > 0.999$), and limits of detection and limits of quantification are in the range from 0.7 and 3 mg/L and 5 and 20 mg/L, respectively.

The short analytical run time leads to low costs and a fast chromatographic procedure. In our method, there no need for esterification of fatty acids unlike the standard method which uses boron trifluoride-methanol as esterification reagent. Both methods gave similar results, but this new method has the advantage such as speed, safety and better recovery rates.

PO-31

Migration of bisphenol and their derivatives from food contact material by target and untargeted analyses

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Food contact materials (FCM) are potential sources of many chemical food contaminants. Recently, the stringent regulations on the use of Bisphenol A (BPA) in a number of consumer products have prompted the industry to seek alternatives to BPA in various applications. At the same time, the information available on the migration of these BPA alternatives and derivatives into beverages and food and especially their potential transformation products (TPs), potentially generated by normal use, is scarce.

To address these threats, we studied the migration of bisphenols, bisphenols diglycidyl ethers and their derivatives from FCMs including plastic bottles, reusable plastic bottles and cans to beverages under various conditions. The migration/leaching tests proposed by the Commission Regulation (EU) 2016/1416, were performed for 10 days at 60 °C or 3 times for 24 hours at 70 °C with different simulants; 20 % or 50 % ethanol, 3 % lactic acid and methanol. After the simulant extraction and derivatisation, the gas chromatography coupled to triple-quadrupole mass spectrometry (GC-MS/MS) was exploited for the quantitative determination of 18 bisphenols (22BPF, BPAF, 24BPF,

BPF, BPE, BPA, BPB, BPZ, BPS BPC2, BPC, BPM, BPPH, BPP, BPBP, BPAP, BP26DM and BPFL). On the same extracts, untargeted analyses were performed by liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (LC- QTOF-MS) in data dependent acquisition mode. Data-analysis included two parallel workflows; a suspect screening using a database generated from the literature, and a non-targeted screening using the MZmine software combined with the R statistical software.

The presence of BPA in all samples, up to 8 µg/L under harsh conditions, bisphenol F in cans up to 1 µg/L and its two isomers (ng/L) was confirmed using target and untargeted analyses. Despite the fact that the identification of relevant features is difficult to achieve due to a large number of mass features generated from untargeted analysis, we observed a time trend for the migration of chemicals from reusable bottles (decrease in a number of mass features from day one to day three) and confirmed that migration of compounds from cans is the most intense and consequently of highest concern. The obtained migration trends enable us to set up a detailed experiment for future studies so that the obtained new pieces of information could better address the problems surrounding the migration of chemicals from FMC.

PO-32

Bisphenols removal in algal wastewater treatment bioreactors – future prospects of product reuse

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Bisphenol A (BPA) is a known endocrine disrupting compound (EDC) that is used in the production of plastics and resins and has been the focus of public concern over recent years. Alternatives to BPA, collectively known as bisphenols (BPs), are now increasingly under the spotlight since they share the same basic structure as BPA and are suspected EDCs. Algal wastewater treatment may present an opportunity to treat wastewater while recycling nutrients at the same time. Reclaimed water could be used for agricultural irrigation and algal biomass as a possible feed supplement, fertilizer or biofuel feedstock. Such reuse will add to the circular economy, with minimal amounts of nutrients and pollutants released into the environment. However, such applications have obvious risks including exposing our food supply to hazardous pollutants and endangering animal and human health. Only with the certainty that reclaimed water and biomass are safe to use, we may proceed with their application in agriculture. In the present study, we aim to investigate the removal of 17 BPs in laboratory-scale algal bioreactors. Algal bioreactors were operated in batch mode under fluorescent grow lights for 9 days. Bioreactors were shaken at 150 rpm and cell count, temperature, pH, conductivity, oxidation-reduction potential and O₂ saturation were regularly monitored. Total nitrogen and total phosphorus were measured at day 0 and day 9. Samples were centrifuged and fil-

tered, residual BPs were extracted using Oasis Prime HLB SPE cartridges. Samples were then derivatized with N-Methyl-N(trimethylsilyl)trifluoroacetamide and the catalyst pyridine and subsequently analysed with GC-MS. We observed an approximately 8-fold increase in algae cell count (3.2×10^6 cells/ml on day 0 to 2.6×10^7 cells/ml on day 9). Relative removal of BPs from water ranged from $9.4 \pm 3.9\%$ (BPS) to $98.5 \pm 0.1\%$ (BP26DM), depending on the compound. Removal of compounds positively correlated with partition coefficient K_{ow} , indicating that adsorption and/or bioaccumulation are likely key processes for compound removal from the water phase. Our future work includes the development of a method for the extraction of BPs residues in the algal biomass to quantify the amount of BPs accumulated and the distribution (mass balance) of BPs residues between the aqueous phase and biomass. We also plan to improve the removal of recalcitrant BPs with a bacteria-algae co-culture. Finally, we would like to assess the bioaccumulation of BPs in tomato fruit.

PO-33

Effects of soil properties, management practices and rice genotypes on Arsenic and Cadmium accumulation in rice

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This work is part of a three-year project (2016-2018) carried out in rice fields located in Pavia Province, in the main Italian rice area (North of Italy). The aim is to assess the influence of soil properties, genotypes and management practices of water and fertilizers on total arsenic (tAs), inorganic arsenic (iAs) and cadmium (Cd) in rice.

Cadmium and As have different behaviour in soil (e.g., while flooding increases arsenic concentrations in rice grains, the level of cadmium decreases, and vice versa in aerobic conditions). It is therefore challenging to simultaneously mitigate both As and Cd accumulation in rice grains, particularly at farm scale, with great variability of soils and agronomic practices.

During the first year of the project, 94 soils and rice grain samples from 3 farms were analyzed to select the fields for the experiments. Total As and As species in rice grains was analyzed by HPLC-ICP-MS; total As and Cd in soils and grains by ICP-MS.

The experimental design was planned to consider one variable at a time:

- Soil effect: 6 soils with different characteristics and As and Cd content;
- Water management effect: 4 water managements (wet or dry seeding and permanent flooding or dry from internode elongation to booting);
- Fertilization effect: 3 fertilization practices applied at one level (urea; lime + urea; ammonium sulfate);
- Genotype effect: 9 genotypes (4 long grain B and 5 round grain).

The As content in the rice grain samples was not related with any single soil characteristic except for a weak link between inorganic As in rice and soil total As. An expected reduction of As has not always been obtained in the dried trials. The soil hydraulic characteristics play an important role in the success of dry period. The use of particular fertilizer, like lime, is confirmed to counteract the accumulation of cadmium, while more experience is needed to understand the role of sulfate in relation to arsenic. The rice varieties show different behaviour that could be attributed to genetic effects determining a greater or lesser ability of the plant to absorb and translocate the different forms of arsenic from the soil solution.

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PO-34

Stability of phenolic compounds during simulated in vitro gastrointestinal digestion of wild raspberries

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Wild raspberries (*Rubus ideaus* L.) are a member of the Rosaceae family and have long been collected and consumed worldwide, especially because they are recognized for their possible health benefits [1].

Changes in the concentration or structure of chemical compounds in plant foods during their digestion could cause different bioactivities in vivo compared to results obtained from in vitro studies. The objective of this research was to determine the effects of an in vitro digestion on phenolic compounds identified in wild raspberries because the number of ingested compounds does not always reflect the amount that is available to the body [2]. Changes in phenolic compound contents in samples obtained at different times of in vitro simulated digestion of fresh raspberries samples were analyzed by RP-HPLC. There were two phases of this simulation, including the gastric phase and the intestinal phase. Digested raspberry samples for HPLC analysis of phenolic compounds were taken 0, 1, 2, 3 and 4 h after the experiment started. The concentrations of gallic acid, cyanidin-3-glucoside, cyanidin-3-sophoroside, catechin and ellagic acid in gastric and intestinal fluids during the simulated digestion were measured and their changes observed. The results obtained at the end of the simulated in vitro digestion showed a significant increase of gallic acid concentration compared to its concentration after 1 hour (gastric phase) of digestion time. Considering all of the compounds that were tested, the most affected compound was flavonoid catechin, followed by ellagic acid. Concentrations of these two compounds significantly decreased at the end of the simulated in vitro digestion (intestinal phase) compared to their concentrations after 1 hour of digestion time. Anthocyanins cyanidin-3-glucoside and cyanidin-3-

sophoroside were quite stable during simulated in vitro digestion. The amount of cyanidin-3-sophoroside during digestion process was in the range of 4.18 – 5.65 mg/100 g of fresh fruit weight, while the released concentration of cyanidin-3-glucoside from raspberry fruit was in the range of 26.68 – 33.87 mg/100 g. Ellagic acid was detected only after 1 h of digestion and its concentration decreased approximately by 50% at the end of the intestinal phase of digestion.

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PO-35

Comparison of algae dietary supplements: antioxidative potential and origin

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There are numerous available dietary supplements based on algae, and their effectiveness and origin are more or less unknown due to weaker regulation in comparison with food and pharmaceuticals. This research was focused on comparing different algae supplements obtainable on Slovenian market (*Chlorella*, *Spirulina*, *Aphanizomenon flos-aquae* and multicellular brown algae) by determining their effectiveness and origin. Antioxidative potential was determined by DPPH radical scavenging method, total phenolic compounds by Folin-Ciocalteu method, elemental composition by X-ray fluorescence (XRF), trace elements by inductively coupled plasma mass spectrometry (ICP-MS) and ratios of stable isotopes of light elements (carbon, nitrogen and sulphur) by elemental analysis isotope ratio mass spectrometry (EA-IRMS). Supplements containing *Aphanizomenon flos-aquae* (AFA) contain higher amount of total phenolic compounds and stronger antioxidative potential than *Chlorella* and *Spirulina* supplements, whereas brown algae supplements have almost none. These results are consistent enough that they enable rough identification of algae in the supplement based on total phenolic compounds and antioxidative potential alone. However, certain samples have higher antioxidative potential, which is caused by manufacturer's addition of extracts and vitamins to enrich the product. Results of iron content, determined by both XRF and ICP-MS, show lower values than declared on the supplements' labels. Exception was one sample, which had double the iron content compared to the declared value on the label. Brown algae samples are rich in iodine, which is their main advertised substance. Other algae samples had iodine content below limit of detection of XRF. Heavy metals (mercury, cadmium and lead) in samples, determined by ICP-MS, are only found in trace amounts and as such do not represent risk to consumers' health. Higher amounts of sil-

icon and titanium in certain samples are probably caused by addition of undeclared compounds for enhancement of the product. All samples of AFA have almost identical isotope ratios of C, N and S, which was expected as all these algae supplements were supposedly gathered from the same source, Klamath Lake in Oregon. It should be noted that safety of such supplement is not fully evaluated by my research, as the AFA is a known toxin producer. Other algae supplements have inconsistent isotope ratios of C, N and S, which I presume is mainly due to different environment and sources of nutrients used for growing these algae. Algae supplements' prices do not seem to reflect their effectiveness.

PO-36

Uranium radioisotopes and Po-210 content in soil and cabbage (*Brassica oleracea*) samples collected in the vicinity of a former uranium mine

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Naturally occurring radionuclides (NORM) are wide spread in the Slovenian environment. One of the areas with elevated levels of uranium and its decay products in soil is the region around Žirovski vrh, where uranium ore processing took place. The Žirovski vrh uranium mine was in operation between years 1984 and 1990. The remediation process is almost finished. Taking into the account the fact that the activity concentrations of uranium and its decay products in some parts of the Žirovski vrh area are naturally elevated it is of a great interest to determine the uptake of radionuclides into plants - especially in the plants which are meant for human consumption. And since is well known that all food and foodstuffs contain naturally occurring radionuclides originating from uranium and thorium decay chains, the knowledge of their activity concentrations is therefore necessary in radiological risk assessment, since a dose coefficient is always related to a specific radionuclide. Due to the short half-life of 138,4 days, Po-210 is one of the radionuclides with the highest radiotoxicity and one of the important sources of the internal radiation dose received by humans..

In the presented work we focused on determination of uranium radioisotopes (U-238 and U-234) and Po-210 content in soil and cabbage samples collected in the vicinity of the former Žirovski vrh uranium mine. All together 17 samples of soil and cabbage were collected in pairs from local producers. The cabbage was chosen due to its long growth period and consequently possible higher soil-to-plant transfer of radionuclides. The locations of sampling were chosen systematically to illustrate the conditions near the mine, a few kilometres from the mine and then compare the collected results with a reference location. The activity concentrations of U-234 in the soil samples were between 12 Bq/kg and 85 Bq/kg while the cabbage samples contained between 0.05 Bq/kg in 0.97 Bq/kg of U-234.

The activity concentrations of U-238 in the soil samples were between 11 Bq/kg and 70 Bq/kg while the cabbage samples contained between 0.04 Bq/kg and 0.5 Bq/kg of U-238. The activity concentrations of Po-210 in the soil samples were between 55 in 144 Bq/kg while the cabbage samples contained between 0,1 Bq/kg and 1,1 Bq/kg of Po-210.

PO-37

Proposal for a european metrology network on food safety - EMN-FS

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Safe and high-quality food is a fundamental prerequisite for human health. Food production has evolved considerably during the past decades in many aspects. The objective of the EU food policy is to preserve the safety of food and to verify alteration and contamination in compliance with the precautionary principle of the Sanitary and Phytosanitary Measures agreement of WTO. By combining the metrology approach with the needs identified by the European Reference Laboratories (EURLs) and National Reference Laboratories (NRLs), a common vision can emerge to face the food-related challenges. A European Metrology Network on Food Safety – EMN-FS is needed to improve the reliability of measurement results along the food chain and to support the efforts in ensuring traceability to SI units in European food control. The overall objective of the proposal is to develop a European network among the metrology community, the standardization and the normative bodies and the EURLs/NRLs who have to deal with the Regulation (EC) 178/2002 laying down the general principles and requirements of food law of food safety and with Regulation (EC) 2017/625 on official controls and other official activities performed to ensure the application of food and feed law. This metrological network will deal with the harmonization of measurements and standard reference procedures, the conduction of measurement comparisons and the development of reference materials, used to control the safety of provided food in order to increase trust among consumers. The EMN-FS will focus not only on the food for human consumption but also on animal feed, thus enhancing animal health and welfare.

The network will further support the standardisation organisations, such as CEN and CENELEC. Solid coordination among all involved bodies can improve the current state of the art in food-related measurements and support the constant improvement of the legislation at the European level. Moreover, it will contribute to a reduction of costs deriving from the dispute and unnecessarily repeated measurements.

PO-38

Moniliformin determination by ligand chromatography using lanthanide ions

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A reliable ligand chromatography analysis was devel-

oped for the determination of moniliformin (MON), an emerging mycotoxin with low molecular weight, primarily produced by *Fusarium* fungi and occurring predominantly in cereal grains. Recently, the EFSA CONTAM Panel recommended the collection of more occurrence data on MON in foods and feeds. Analytical methods for MON determination in food have been mostly based on HPLC-UV or LC-MS/MS. However, because of its ionic nature, MON is weakly retained by reversed-phase chromatography and the separation may be tricky. MON separation is mainly carried out using ion pair formation or hydrophilic interaction liquid chromatography; recently, specific columns for polar compounds were used. In this work, it was evaluated if a new approach based on the ligand chromatography could be suitable to obtain a satisfactory separation. Besides its ionic nature, MON is a α -di-ketone; these organic compounds, mainly β -di-ketones, can easily form complexes with lanthanide ions. Then, it was evaluated if the use of a lanthanide ion solution, added in the chromatographic mobile phase, can improve the separation of MON. A RT amino column was used, since it strongly retained MON (no peak was observed until 30 min.) when water-acetonitrile 1+1 was used as mobile phase. Substituting the mobile phase with acetonitrile:10 mM $\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$ (or $\text{TbCl}_3 \cdot 6\text{H}_2\text{O}$) 2+8 v/v, a satisfactory and fast MON elution was obtained. Four MON calibration standards were injected (500, 1000, 2500, and 5000 $\mu\text{g l}^{-1}$) showing a satisfactory calibration curve ($R^2=0.998$). Different lanthanide concentrations in the mobile phase (from 1 to 50 mM) were tested; the MON retention time increased diluting La^{3+} or Tb^{3+} concentration. These results can indicate the formation of a complex between lanthanide ions and MON; the formation of a complex $[\text{La}(\text{MON})_3]$ increases the affinity of MON for the mobile phase, resulting in a faster elution from the column. A satisfactory chromatographic separation and an accurate determination by UV or MS/MS (1mM lanthanide ion) was obtained. Finally, a suitable extraction and purification method for MON determination in cereal samples was developed.

PO-39

Targeted healthy and undesirable compounds in small and large scale brewed beers

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Determination of targeted healthy and undesirable compounds in the most popular small and large scale brewed beer sold in Italy was carried out. Folic acid and the main folates, pyridoxine, total phenolic content and antioxidant capacity were quantified; moreover, amines and mycotoxins were determined to evaluate if correct practices of processing were carried out. The vitamin 10-formyl-folate, quantified by LC-MS/MS, was the main folate in all beer samples. Total folate concentration ranged from 15.5 in a large scale (LS) lager beer to 104.8 $\mu\text{g/l}$ in a small scale (SS) wheat beer, showing a mean of $54.0 \pm 19.7 \mu\text{g/l}$; for LS and SS beer, the average values were 46.4 ± 15.3 and $61.4 \pm 20.8 \mu\text{g/l}$, respectively. Mean

level of folates was higher in red and wheat beer than regular (light ale and lager) beer (average value: 74.3 ± 15.7 vs 69.0 ± 18.4 vs 47.8 ± 15.2 $\mu\text{g/l}$, respectively). For pyridoxine (vitamin B6), the highest level was determined by LC-MS/MS in a SS red beer, while the lowest in a LS Italian lager beer. High variability was observed among the samples; the 42.8% and 39.0% of SS and LS beers, respectively, showed a pyridoxine level lower than 100 $\mu\text{g/l}$, while 14.3% and 19.5% a level higher 200 $\mu\text{g/l}$. Average total phenolic content (TPC) was 526 ± 157 and 423 ± 111 mg/l for SS and LS beer, respectively; the antioxidant capacity 1.6 ± 0.5 and 1.1 ± 0.4 measured as mM Trolox and 5.5 ± 1.8 and 4.0 ± 1.1 measured as mM Fe^{2+} . TPC and antioxidant capacity can be influenced by beer style, in agreement with literature works. In Italy, legislation bans pasteurization and filtration process for SS beer; the higher values for SS beer can be due to the fact that pasteurization, as any other thermal treatment, can decrease the TPC and antioxidant capacity. For undesirable substances, low contamination was found for ochratoxin A, deoxynivalenol, citrinin and sterigmatocystin both for LS and SS beer samples. The highest level of amines, determined by LC-MS/MS, was quantified in a SS red beer, while the lowest in a SS Italian lager beer; only in 7 samples, amines exceeded 10 mg/l . Putrescine was the most abundant, while histamine was always lower.

PO-40

Effect of simulated gastrointestinal digestion on phenolic compounds in *Lythrum salicaria* L. extracts

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Aromatic and medicinal plants are an integral part of the human diet and an important source of phytochemicals associated with reduced risk of chronic diseases. For example, phenolic compounds are recognized for their antioxidant, antibacterial, antiviral, anti-inflammatory and anti-carcinogenic activities [1]. In order to exhibit a positive effect on human health, polyphenols from plant food have to be released (bioaccessibility) and absorbed from intestine [2]. Thus, in vitro digestion models are commonly used for determination of the bioavailability of plant phenolics in order to evaluate their potential as a functional ingredient.

Purple loosestrife – *Lythrum salicaria* L. (Lythraceae) is a perennial herbaceous native to Europe, Asia and Northern Africa. It has been used for centuries in European traditional medicine and the current studies showed that this plant possesses antidiarrheal, antimicrobial, antioxidant, anti-inflammatory and anti-diabetic activities. *L. salicaria* contains a high amount of polyphenols, including C-glucosidic ellagitannins and C-glucosidic flavonoids as dominant compounds. For better understanding of the biological significance of examined plants, simulated in vitro digestion studies were performed on *L. salicaria* aerial part (LSA) and root (LSR) methanol extracts. The changes in the content of phenolic compounds identified in the extracts were assessed using a simulated oral,

gastric and small intestinal model. The concentration of individual phenolic compounds in the digested extracts was determined by HPLC analysis every hour during 4 h of digestion time.

HPLC analysis showed that both extracts contain gallic acid, ellagic acid and three flavone C-glucosides, orientin, isoorientin, and vitexin. All compounds detected in methanol extracts were quite stable during in vitro gastrointestinal digestion. The highest concentrations of all detected phenolics in LSR were detected after 2 h of simulated digestion (gastric phase), while during the intestinal phase their concentrations slightly decreased. The changes in quantities of identified flavone C-glucosides, gallic and ellagic acid were less pronounced in LSA during the digestion process compared to LSR. Obtained results suggest that gallic acid, ellagic acid, orientin, isoorientin, and vitexin from *L. salicaria* extracts may be absorbed unchanged during digestion and thus achieve potential health benefits.

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PO-41

Liquid chromatographic-tandem mass spectrometric method for determination of tetrodotoxin in mussels

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Certain bacteria, fungi, vertebrates, or marine microorganisms are able to synthesize a large group of toxins that can accumulate in fish and shellfish. When people consume such contaminated seafood, depending on the toxins, the symptoms can be diarrheic, paralytic, amnesic, and neurologic. Tetrodotoxin (TTX) is naturally-occurring toxin produced by marine bacteria. It is well known in the Southeast Asia region to cause many serious food poisoning following ingestion of contaminated puffer fish (fugu) [1]. It can also be accumulated in others edible marine species such as gastropods and bivalve molluscs. TTX and its analogues are not monitored on regular basis in the European Union yet, but they were found in mussels, Pacific oysters and clams from UK, in mussels from Greece and NW Spain, in mussels and oysters from Netherlands and in also shellfish (mussels and clams) from Italy (Sicily and NE Italy) [2, 3]. At the institute we monitor the presence of marine biotoxins since 1993. HPLC-FLD method is used for determination of paralytic, HPLC-DAD for determination of amnesic and LC-MS/MS for lipophilic toxins. Because the presence of TTX is reported in the vicinity of Slovenia, the aim of our study was to develop a method for determining TTX in bivalve mussels. We slightly modified and optimized the sample preparation

procedure, that was described elsewhere [4]. TTX was extracted from mussels into acetic acid (1% aqueous solution) in a boiling water bath. After the protein precipitation with the addition of ammonia (25% aqueous solution), the extract was purified on the Supelclean ENVI-Carb SPE columns (500 mg/6 ml). The TTX content in the extract was determined by LC-MS/MS (Waters Alliance 2695 in Quattro Micro API, Micromass) under isocratic elution with a Kinetex HILIC column (2.6 μ m 100 A, 150 x 2.1 mm, Phenomenex). Matrix matched calibration curve was used and the method was linear in the range of 100 μ g/kg-10 mg/kg. The trueness of the method was verified with participation in proficiency test organized by European Reference Laboratory for Marine Biotoxins.

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PO-42

Validation of a confirmatory multi-residue method for the determination of thirteen hormones in several bovine matrices using HPLC-MS/MS

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Synthetic and natural hormones are used in the production and intensive breeding of animals as growth factors and reproductive regulators. However, the illegal and inappropriate use of these substances increases the risk of introducing residues into the food chain. Based on their carcinogenic and teratogenic effects, the European Union has banned the use of these substances since 1985. For this reason, the development of sensitive and reliable analytical methods for the monitoring of these residues in food has become a necessity to define and control the quality of products. A liquid chromatography analysis method coupled to tandem mass spectrometry and based on Quenchers extraction has been developed to analyze thirteen synthetic and natural hormones in different bovine matrices. In order to study the reliability of this method, its validation has been carried out in the selected matrices (liver, kidney, bile, and hair) according to European Decision 2002/657 / EC. The method demonstrates good linearity ($R^2 > 0.99$) as well as accuracy with coefficients of variation for repeatability and reproducibility lower than 23%. The values of CC α and CC β were determined for each analyte indicating values ranging from 0.13 to 0.86

μ g/kg and 0.25 to 1.72 μ g/kg, respectively, for most of the analytes. Higher values were obtained for 17 β -estradiol, estriol, 17 α -ethinyl-estradiol ranging from 1.73 to 13.95 μ g/kg for CC α and from 3.47 to 23.87 μ g/kg for CC β . The recovery rate in the different matrices (liver, kidney, bile, and hair) varies from 51.5 to 107%. The matrix effect of the method was also evaluated and indicated significant suppression values for the liver and the kidney, which varied, respectively, from -45 to -15.5 % and -35 to -2.5%. In the same way, the other two matrices hair and bile show lower matrix effects than the others. Finally, this method has been successfully applied to detect anabolic hormones in ninety-one samples (muscle, liver, kidney, bile) collected from different local butchers. As results, progesterone was found in 44 samples at concentrations 0.11 to 11.7 μ g/kg, while testosterone was detected in 41 samples at concentrations ranging from 0.5 to 9.52 μ g/kg.

PO-43

A new method for determination of monomethylmercury based on separation of Hg dithizonates and cold vapour atomic absorption detection

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Mercury and its chemical compounds are extremely hazardous and can exist in a large number of different physical and chemical forms with wide range of properties. To understand mercury chemistry in natural systems analytical techniques that allow determination of total and major species of mercury in air, water, sediment and biota have been developed. New developments are primarily focused on high tech equipment, including mass spectrometry that allows the use of stable isotopes for method development and validation but also to trace the source of mercury and its compounds using multicolector mass spectrometry (MC ICP MS).

However, methods based on mass spectrometry and other currently available instrumental techniques for speciation are based on principles that require clean laboratory conditions, expensive gases and a lot of experience. For most routine laboratories such conditions are unsuitable which results in the fact that speciation analysis are only done in expert laboratories. Moreover, recently adopted Minamata convention calls for simplified and "easy-to-use" methods that allow speciation of mercury world-wide.

Simple extraction method that can be used to measure monomethylmercury (MMHg) in biological and environmental samples has been developed. The method is based on the formation of Hg dithizonates and their extraction into toluene. Hg(II) and MMHg dithizonates are then separated on silica gel column and selectively collected into separate vials followed by digestion with mixture of acids (HNO₃/HClO₄ and H₂SO₄) and measured by CV AAS. The method separates inorganic and organic mercury quantitatively in biological and environmental samples. Separation is very good also in samples where the percentage of MMHg is low. The results obtained were ver-

ified by the analysis of Certified Reference Materials and comparison of the results obtained by alternative methods based on ethylation and CV AFS detection. The average repeatability and reproducibility of the method are 1.5% and 4.3%, respectively. Expanded relative standard uncertainty according to Nordtest is 12.8% using coverage factor $k=2$.

Compare to other methods this method is simple and inexpensive. The instrumentation that is used for total mercury measurements can be used to measure organomercury compounds. In our laboratory we use semiautomated AAS that for its operation has diaphragm pump to pump the air and doesn't use any expensive gasses. The laboratory ware is made of glass which is easy to get in most of the countries and is easy to clean for mercury analysis.

PO-44

Analysis of titanium dioxide nanoparticles in food by ICP-MS/MS in single particle mode

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In the European Union, engineered nanomaterials should be labelled as ingredients in food in accordance with Regulation 169/2011. This poses several analytical challenges in relation to sample preparation, the limitations of existing analytical techniques and the lack of validated studies and reference materials. In that regard, we investigated food samples containing titanium dioxide (TiO₂) as the white color additive E171. Although the white pigment of E171 is achieved when the particles are not in the nano size, a certain fraction of nanoparticles (NPs) can still be present in the food due to their broad size distribution.

One of the most commonly used technique for the detection and characterization of metal-based NPs in food is inductively coupled plasma-mass spectrometry (ICP-MS) in single particle mode (spICP-MS). We applied spICP-MS technique for the analyses of TiO₂NPs in chewing gum, chocolate candy and cake frosting (inscription). Further, we spiked reference TiO₂NPs to milk as an example of a calcium-rich matrix. For this purpose, spICP-MS analyses were performed on Agilent 8900 ICP-QQQ-MS in MS/MS mass-shift mode (m/z 48 \rightarrow m/z 64) with the use of O₂ and H₂ reaction gases in order to overcome isobaric interference of calcium on m/z 48.

Repeatable determination of number-based particle size distributions was achieved with a minimum detectable particle size of ~30 nm. The median particle diameters were in the range of 130 to 200 nm and particle mass concentrations were in the mg/g range. In addition, similar size distributions for TiO₂NPs in different foodstuffs studied were observed by asymmetric field flow fractionation (AFFF) coupled to ICP-MS.

We showed that relatively easy implementation of spICP-MS in state-of-the-art ICP-MS instruments makes it a promising technique for routine analysis of titanium dioxide nanoparticles in food samples with high calcium content.

PO-45

Biodegradable food packaging materials containing substances of plant origin

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Polymers are commonly used in the production of packaging, intended for contact with food products. The purpose of food packaging is to protect food products during transport and storage, as well as preserving the quality of food. The packaging fulfils four basic functions: protection, communication, convenience and containment.

Polymers replace traditional materials such as glass and paper due to their low price and specific properties. The use of polymeric materials of petrochemical origin, generates waste, which disposal is difficult. Legislation and pressures on the use of environmentally friendly materials have increased interest in biodegradable polymers and eco-friendly processing additives. According to current trends an important group of packaging materials are biodegradable polymers and additives of natural origin.

The combination of biodegradable polymers with substances of plant origin allows obtain a new generation of packaging materials. Such packages, which are fully composed of natural substances, are relatively easily decomposable, friendly to both the environment and the health of consumers.

During presentation, biodegradable polymeric materials based on polylactide (PLA) and polyhydroxybutyrate (PHB) with plant polyphenols will be shown. The influence of the addition of natural polyphenols on the properties of polymers and the stabilization of biodegradable polyesters will be presented.

PO-46

“Green” nanotechnologies: Synthesis of silver nanoparticles using *Lythrum salicaria* L. extracts and study of their biological activity

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Silver nanoparticles (AgNPs) are widely used in various fields; including applications in antimicrobial products, cosmetics, biomedical and drug delivery purposes, and biological labeling. [1] Use of AgNPs in food packaging has recently attracted much attention. It is important that less toxic chemicals should be considered for production of AgNPs when those NPs are to be used in medical applications or food packaging. In that regard, new biological methods of AgNPs synthesis have been developed. In this study, a method for simple and environmental friendly biosynthesis of silver nanoparticles using an aqueous extract of the aerial part (LSA)

and root (LSR) of the plant *Lythrum salicaria* (fam. Lythraceae) was developed. The formation of AgNPs during synthesis was observed by visual color change and UV-Vis spectrophotometry. The LSA- and LSR-synthesized nanoparticles were characterized by scanning electron microscopy with energy dispersive spectroscopy (SEM/EDS), X-ray powder diffraction (XRPD) and fourier transform infrared spectroscopy (FTIR). Antioxidant and antimicrobial activities of obtained AgNPs were determined.

Obtained results showed a highly efficient inhibitory effect of synthesized AgNPs on both Gram-positive and Gram-negative bacteria, as well as yeast *Candida albicans*, with the minimal inhibitory concentrations (MICs) between 1.25 and 0.156 mg/mL. LSA-AgNPs showed higher antimicrobial potential than LSR-AgNPs. According to the results of the antioxidant activity, LSA-AgNPs displayed higher potential in the neutralization of ABTS+ (IC₅₀ 127.78 µg/mL) in comparison with LSR-AgNPs (IC₅₀ 141.66 µg/mL), while synthesized AgNPs were almost inactive towards DPPH scavenging. HPLC analysis of *L. salicaria* extracts used for AgNPs synthesis showed that both LSA and LSR extracts contained ellagic acid and flavone C-glucosides orientin, isoorientin, and vitexin. These identified compounds may contribute to the formation of AgNPs, acting as excellent reducing agents because of their abundant hydroxyl groups, and powerful dispersants (owing to their derived carbonyl groups), which enhanced the NPs' stability. The findings of this study suggest that *L. salicaria* could be used for the environmentally safe biosynthesis of AgNPs with promising antimicrobial properties.

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PO-47

Antimicrobial activity of molybdenum oxide nanoparticles and their potential for application in food industry

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Ensuring food safety and reducing economic losses due to food spoilage and deterioration are priority in today's globalizing food market with worldwide transportation and consumption of minimally processed foods. Every year, approximately one third of all produced food ends as food waste [1], to which is also contributed by microbial food spoilage. Microbial contamination of food can occur during primary production, transport or processing, or later during distribution of final products. Therefore, it is important to look for new, innovative ways to reduce microbial contamination of food.

Recently, it was found that MoO₃ particles express a pronounced antibacterial effectiveness [2,3], which depends on surface morphology and crystal structure. We produced two different MoO₃-x nanoparticles with

orthorhombic crystal structure but different morphology, size and shape. Many authors reported, that MoO₃ nanoparticles in an aqueous medium form molybdic acid [2,4,5]. It is well known that the acidic environment slows down or/and prevents bacterial and fungal growth [2,5]. The antifungal activity of the MoO₃ against *Candida albicans* and *Aspergillus niger* was also reported [5].

We will present chemical and physical characterization (UV-Vis spectroscopy, scanning electron microscopy-SEM, BET, zeta potential, pH curves) of two different MoO₃ nanoparticles, and their antimicrobial activity against important food-related microorganisms, which can present a persistent source of cross-contamination of the products, causing food spoilage and presenting potential health risk for consumers. As such, we included *Staphylococcus aureus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus flavus* and *Penicillium verrucosum* in our research. Finally, we will present the possibilities of using this materials as part of food-contact surface coatings or incorporated in innovative packaging materials.

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PO-48

Development of an efficient passive sampler adsorbent for the detection of mercury in water via stratified nanostructured knitting

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This work reports the development of a nanostructure material based passive sampler, which binds trace mercury (Hg²⁺) by employing ultrathin graphitic carbon nitride (g-CN) nanosheet as effective adsorbent. The g-CN nanosheets were obtained by exfoliating the bulk g-CN which was synthesized via a thermal polycondensation process. The as-prepared nanosheets were characterized using different analytical techniques, which confirmed graphite-like structure. The results showed high recovery capacities for Hg²⁺ in different matrices in the following order: Sea < River < Rain < Mili Q water of 89%, 93 %, 97 and 100 %, respectively. Ion interference studies (Co²⁺, Ca²⁺, Zn²⁺, Fe²⁺, Mn²⁺, Ni²⁺, Bi³⁺, Na⁺ and K⁺) were also performed to check the specificity and selectivity of g-CN towards Hg²⁺. There was minimum or no effect of the presence of ions on the binding efficiency of Hg²⁺ on g-CN nanosheets. The effect of pH (2.4, 4, 7 and 9) on the binding efficiencies of Hg²⁺ on g-

CN was also studied. It was found that g-CN nanosheets showed enhanced binding response to Hg²⁺ in comparison to its bulk counterpart, which could be ascribed to the strong affinity between g-CN and Hg²⁺ through its -NH and -NH₂ groups. This allows detection of Hg²⁺ in aqueous solutions with high sensitivity and selectivity. A mercury analyzer used in present work to quantify Hg²⁺ retained on g-CN and supernatant. Such a sampling material reported an efficiency of adsorption that was equal to ~99%. Temperature and relative humidity only mildly affected the material performances. These defined nano-interwoven structures “knitting” seem to be promising candidates for mercury samplers. The nano-knitting structures seem to be promising candidates for mercury samplers, due to the strong affinity with Hg²⁺, and the wide adsorbing surface. These results demonstrated that the g-CN was a potential material for detecting Hg²⁺ in water. The g-CN can be tailored and tuned which then be used in clinical, environmental and industrial applications.

PO-49

Are there titania nanoparticles in my food?

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The health risk of consuming engineered nanoparticles has not been resolved yet. This is mainly due to the facts that the particles of concern are extremely small, usually present in food in very small quantities and therefore difficult to find. In addition, it is difficult to assess their real effect on human health.

Titania, TiO₂, is probably the most abundant engineered inorganic ingredient in food. In relatively large, however, still very small amounts it is present, for example, in chewing gum crust. According to the analysis of titania particles extracted from chewing gum, the crystallites are in the form of anatase with the size of 100-200 nm. This approximately equals the size of food-grade titania powder available in the market for cake decoration. Both powders are highly agglomerated in liquids, resulting in mean hydrodynamic diameter well exceeding the definition of “nanoparticles”. On the other hand, a small portion of the extracted particles was found to have a diameter below 100 nm, and similar was observed for the cake decoration titania powder. This suggests that even though the mean particles size does not qualify the powder as a nanomaterial, it should be considered as potentially hazardous.

Besides the particles intentionally added to food for various purposes (colour, crunchy taste, etc.), inorganic particles can also migrate from food contact materials into food during food processing. In this case, the particles are not necessarily food-grade. The analysis of the “quasi-ceramic” non-stick pan coatings are sources of such migration. Several coating, however, confirmed that the titania particles embedded into a silicone-based matrix are in the rutile form within the size range of 100 – 250 nm with approx. one third of particles below 100 nm. The analysis also revealed that migration of the particles is possible

into acidic foods and due to matrix degradation.

PO-50

Fast analysis of alpha-tocopherol in olive oil by Paper Spray tandem mass spectrometry and isotope dilution method

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The extra virgin olive oil provides an intake of alpha-tocopherols which is part of the vitamin E molecular complex. Paper spray ionization is a simple technique for introducing unprocessed samples of fluids to the mass spectrometer. Analyte ions are generated by applying a high voltage and a few microliters of spray solvent onto a triangle paper. PS-MS/MS experiment in negative ion mode, has been proved to provide excellent results in terms of specificity and sensitivity for a rapid screening of tocopherol in extra virgin olive oil. The MS/MS spectrum of the deprotonated molecule [M-H]⁻ of alpha-tocopherols is characterized by few fragments, the most abundant product ion is the ion at m/z 163, the same ion increased of six Da is present in the MS/MS spectrum of internal standard α -tocopherol-D6. The quantitative method, is based on the coupling of the isotope dilution method and the PS-MS/MS under MRM condition. The selected gas-phase reactions are those described above, in particular the transitions [M-H]⁻ → m/z 163 and [M-H]⁻ → m/z 169 have been monitored for alpha-tocopherol and for internal standard. The average of the ion current of each monitored transition, over the total acquisition time, is used for the quantitative analyses. The quantitative analyses were performed using a calibration curve, the linear regression was made using triplicate samples of five standard solutions at different concentrations of alpha-tocopherol with the same, 8 mg/L concentration, of the labelled internal standard, showing good linearity in the range from 2 to 16 mg. The developed methodology has been applied to real samples of extra virgin olive oil and to spiked samples in order to evaluate the analytical parameters of the approach. The sample preparation is very quick and easy: in fact the oil is simply dissolved 25 times with acetone, after adding the appropriate amount of internal standard. The entire process of analysis is very rapid, in fact the acquisition time of the PS-MS experiment is only 2 min. For all the analyzed samples, the RSD % values were calculated lower than 15 %, confirming the good repeatability of the PS-MS/MS measurements. Furthermore, the calculated analytical parameters of LOD, LOQ, as well as accuracy and reproducibility, confirmed the goodness of the proposed approach.

PO-51

Effect of yeast cells exposure to fermented *Spirulina* on their protein profile: Characterization of yeast lysates using high resolution Orbitrap mass spectrometry

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Cyanobacteria *Spirulina* (*Arthrospira platensis*) is a rich source of nutrients and bioactive compounds with high antiinflammatory and antioxidant activity [1]. Proteins of high bioactive value constitute a large part of *Spirulina* dry weight [2], [3]. Its nutritional and bioactive efficiency can be enhanced using lactic acid fermentation due to transformation of original compounds and new metabolite production [4]. As only little research has been done in this field the aim of this study was to investigate effect of lactic acid fermentation on *Spirulina*'s bioactivity.

Fresh *Spirulina* samples were fermented using *Lactobacillus plantarum* culture. Then water extracts of fermented and non-fermented *Spirulina* biomass were used for yeast cells treatment. Afterwards yeast cell lysates were prepared and Bradford assay was used to determine protein content of the lysates. A comparative proteomic study using a Q-Exactive HF Orbitrap instrument was done for studying protein expression alterations. Label-free liquid chromatography-tandem mass spectrometry coupled to data dependent acquisition was employed to identify and calculate protein fold changes between conditions. After each run, the raw files were qualitatively and quantitatively analyzed by Proteome Discoverer. Proteins were identified against the UniProt database.

Gathered results show that yeast cells exposure to fermented or non-fermented *Spirulina* extracts affects their protein content. Significant differences were found between samples treated with *Spirulina* extracts and control, samples treated with fermented and non-fermented *Spirulina* extracts, and samples treated with different concentrations of *Spirulina* extracts. In the yeast cells treated with *Spirulina* extracts higher expression of proteins involved in amino acid and carbohydrate metabolism and cell transport and catabolism was found. Higher expression of proteins involved in carbohydrate, lipid and amino acid metabolism, genetic information and protein processing and higher C-phycocyanin levels were found in yeast cells treated with fermented *Spirulina* extracts compared to non-fermented *Spirulina* extracts. Additional research will be carried out to specify the effects of lactic acid fermentation of *Spirulina* on its bioactive and nutritional properties.

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PO-52

Toxic elements in whey protein supplements

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The Federal Institute of Metrology METAS works on the development of Certified Reference Materials (CRM) for food safety. In order to develop a new whey protein reference material for the analysis of contaminants, we began a study on toxic elements in different whey protein samples. Whey protein is a supplement largely used for muscle growth, body weight reduction and immune system boosting. In addition to the beneficial effects, whey protein powders could contain toxic elements, such as lead, arsenic, cadmium, mercury, and other contaminants. The present study investigated the heavy metals contamination in thirteen brands of whey protein present in the Swiss market.

The acid digestion of the samples was performed in HNO₃ at 190 °C using a microwave digestion system. Total arsenic, cadmium, chrome, lead, barium and nickel were measured by inductively coupled plasma mass spectrometry (ICP-MS). Analytical determinations of total mercury in whey protein products were performed by direct mercury analysis system.

The results showed that the highest cadmium, nickel and total mercury concentration levels, respectively 48.3 ± 2.4 µg/kg, 1290 ± 64 µg/kg and 0.270 ± 0.013 µg/kg were found in samples containing cocoa. In the same brand samples containing only natural whey protein without flavouring addition, nickel was found in lower concentration, cadmium and total mercury level were lower than LOD. Chrome and barium were also found in higher concentration in whey protein containing cocoa. Lead was detected in one sample with chocolate flavor at concentration level of 47.2 ± 2.4 µg/kg. The concentrations of the toxic elements measured in whey protein samples were below the maximum legislation limit for food supplements. The study will be extended to more whey protein samples. To calculate the effective risk associated with whey protein consumption, factors such as amount consumed, intake level, metal species, length of exposure must be taken in account.

PO-53

Dietary habits of Slovenian mothers and breast milk fatty acid composition: comparison of inland and coastal areas

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Human milk contains high amounts of nutrients essential for the growth and development of babies, w-6 and w-3 polyunsaturated fatty acids (PUFAs) among others; however its quality depends on maternal diet. The objective of the study was to investigate associations between dietary habits and FAs composition in maternal milk samples from two geographically distinct areas of Slovenia: coastal (Koper) and inland (Pomurje). Residents of the two areas traditionally have different dietary habits.

Primiparous mothers from Koper (N=36) and Pomurje (N=38) provided milk samples in the 6 – 8 weeks after delivery, while their dietary habits and life-style data were obtained through self-reported questionnaires. Identification and quantification of FAs in maternal milk samples were performed by in-situ trans-esterification method. The characterization of fatty acids methyl esters was performed by gas chromatography. Database of FAs composition for the two areas of Slovenia for mature milk was established. Associations between variables obtained through questionnaires, especially dietary habits, and FAs composition in maternal milk samples were investigated using descriptive statistic, univariate analyses, simple and multiple linear regression analysis.

Results showed that dietary habits differed between the study areas (inland vs coastal): intake of game (higher in inland), fresh water fish (higher in inland), fresh seafood (lower in inland), frozen seafood (higher in inland). The mean percentages of individual FAs differed statistically significantly between the study areas in the case of 35 FAs: 19 cases had higher percentages in the coastal area, and 16 cases in the inland area. The levels of saturated and monounsaturated FAs in milk were statistically higher in the coastal than in the inland area. On the other hand, PUFAs, w-3, w-6 levels were lower in the coastal than in the inland area, despite higher intake of fresh seafood observed in the coast. The ratio w-6/w-3 levels did not differ significantly among the studied areas.

Since the self-reported data on food intake is difficult to fill in and incorporates high uncertainties, this study provided a good starting point towards establishing more precise biomarkers of dietary intakes.

PO-54

Missing data imputation methods for food composition data

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Missing data is a common problem in most research fields and introduces an element of ambiguity into data analysis. It can arise due to different reasons such as mis-handling of samples, measurement errors, deleted aberrant values or simply lack of analysis. The Nutrition domain is no exception to the problem of missing data. Food composition databases (FCDBs), which are detailed sets of information on the nutritionally important food components (Food composition data – FCD), represent fundamental information resources for Food science. One of the criteria for a good quality FCDB is its tendency towards complete coverage of both, foods and nutrients. Complete FCDBs are of great importance because FCD is used widely and many institutions rely their work on their national FCDBs. Even though there are few national FCDBs that aim towards complete coverage, most of them are far from being complete. Missing value handling is of great importance when providing an efficient and valid data analysis. In the present study, we focus on missing data imputation techniques which are based on methods for substituting missing values with statistical prediction. To find the most

suitable approach, we compared the following statistical prediction methods: fill-in with mean, fill-in with median, Non-Negative Matrix Factorization (NMF), Multiple Imputations by Chained Equations (MICE), Nonparametric Missing Value Imputation using Random Forest (MissForest), and K-Nearest Neighbors (KNN). The data for our experiment was extracted from the national FCDBs of 10 countries, which were collected by EuroFIR (European Food Information Resource Network) and comply with the Food data structure and format standard (BS EN 16104:2012). Using this information, we created four complete datasets with selected nutritional values for foods from four food groups: Potassium in Fruits, Sodium in Fruits, Sodium in Vegetables, and Protein in Meat. Because FCDBs are quite incomplete, obtaining complete datasets was not an easy task, and the datasets obtained are small. The statistical prediction methods for missing data imputation were tested by setting: 10%, 20% and 30% of the data as missing (as separate use cases), then calculating the missing values, and evaluating the results by comparing the obtained values with the actual values. The comparison was done using the evaluation criteria as follows: Mean Absolute Percentage Error, Mean Kullback-Liebler Divergence Error, Root Mean Squared Error, and the execution time. The obtained results suggest that the NMF and missForest methods perform best and deserve further consideration in practice.

PO-55

Proficiency test PT mushroom - trace elements in mushroom

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In the framework of the of the “ERA Chair ISO-FOOD for Isotope Techniques in Food Quality, Safety and Traceability”, we organised a proficiency test (PT) for the determination of trace elements in edible field mushroom (*lat. Xerocomus badius*, family Boletaceae).

The PT Mushroom was prepared from mushrooms collected from a non-contaminated rural area as part of an IAEA project (H. Polkowska-Motrenko, M. Rossbach, Accred. Qual. Assur., 2007, 12:343-350). In summary, whole mushrooms were cleaned, chopped up with a stainless steel knife, air dried, milled and sieved. The fraction of sample with a diameter 1 mm was collected. The whole sample was then milled, using a zirconium planetary ball mill, sieved (125 µm) and homogenized. Care was taken to avoid contamination. After homogenization, the particle size was determined, and tested for homogeneity. The material was then subsampled into HDPE bottles, containing 2 g of sample.

Fifteen laboratories from eleven countries registered to the exercise and reported results for 13 elements. The quality requirement expressed as relative standard deviations for proficiency assessment (σ_{pt}) were set according to modified Horwitz equation: 15% for Ag; 18% for As; 16% for Br; 14% for Cd; 11% for Cs; 8% for Fe; 20% for Hg; 3% for K; 19% for Mo; 6% for Na; 6% for Rb; 18% for Se; 7% for

Zn. Most of the participating laboratories reported satisfactory results (according to the z score) which confirms their overall good performance.

Noticeable deviations are mostly found for toxic elements such as As, Cd and Hg. Speciation data for Hg were reported by two laboratories with significant differences. Speciation of As was not reported. Performance of accredited laboratories was comparable to non-accredited labs. Wet digestion using microwave was practised by most laboratories, but the results were similar to convectional wet digestion techniques. In case of mercury two methods were used, combustion followed by CV AAS method (DMA) and wet digestion followed by CV AAS. Both methods provided comparable results. In case of microwave wet digestion lower results were obtained for Hg, most probably due to losses. For other elements no significant differences were observed among the most commonly used detection methods (ICP MS and NAA).

PO-56

The isotopic approach to the authenticity of Cypriot traditional products

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The contribution of local products to the establishment of the “identity” of a region, aids in the creation of a correlation between the products, the unique characteristics and the geoclimatic conditions existing in the region of production. Recently, the consumers have diverted their interest towards local traditional products, which possess unique quality characteristics.

In the framework of “AGROFOOD” project funded by the INTERREG V-A 2014-2020, the specific characteristics of indigenous Cypriot products were studied and compared to similar products from other regions, in order to differentiate and certify their origin. In the present work, the combined information from isotopic ratios of $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ by IRMS and D/H by SNIF-NMR spectroscopy, has been observed to create a unique isotopic fingerprinting of the products under study.

PO-57

The application of isotopic and other techniques to verify the declaration of apple juices on Slovenian market

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A common theme of food authentication and traceability studies is the requirement for a database of genuine samples to which a sample can be compared to in order to establish its authenticity. The same database can be used for determining geographical origin. The presented research shows how the developed model was used to control authenticity of apple fruit juices in a real-world application on Slovenian market. Thirty-six commercial

apple juices formed the basis of this study. The samples were produced in the nine countries, ten juices were cloudy, nineteen made from concentrate and eight from apples cultivated organically. Eight samples were made from Slovenian apples (as declared on the label, or from local producers). Fourteen parameters were determined in selected samples including: total soluble solids (TSS), titratable acidity (TA), glucose, fructose, sucrose, $\delta^{13}\text{C}_{\text{sug}}$, $\delta^{13}\text{C}_{\text{pulp}}$, $\delta^{15}\text{N}_{\text{pulp}}$, $\delta^2\text{H}$, $\delta^{18}\text{O}$, $\delta^{13}\text{C}_{\text{EtOH}}$, (D/H)I, (D/H)II and R. Commercial juices from apples grown organically or by integrated/conventional methods could not be separated, whereas in a cross validation test 91.0% of the samples are classified correctly. The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in pulp, which are important differentiators of agriculture production practice, were not included in this evaluation, since the pulp was obtained from only eight samples. Further good separation between all three groups (authentic apple juices, commercial apple juices made from concentrate and juices labelled as not being “not made from concentrate”) was achieved using all of the following twelve parameters: TSS, TA, glucose, fructose, sucrose, $\delta^{13}\text{C}_{\text{sug}}$, $\delta^2\text{H}$, $\delta^{18}\text{O}$, $\delta^{13}\text{C}_{\text{EtOH}}$, (D/H)I, (D/H)II and R. Ninety-eight percent of the samples were correctly classified. In LDA, a good distinction between genuine samples and commercial juices labelled “not made from concentrate” was not possible using only stable isotopes. Only 47.1% of samples were correctly classified. Linear discriminant analysis also shows that it is possible to distinguish between Slovenian apple juice and apple juice from other countries, independent of their botanical origin. The total prediction ability was 90.0%. Furthermore the stable isotope data from commercially available apple juices from the Slovenian markets revealed cases of mislabelling on packages. Of the thirty-six commercial samples analysed, three samples (10%) were adulterated with beet sugar. All of these products were declared as being made from 100% pure apple juice.

Acknowledgement: Operation part financed by the European Union, European Social Fund.

PO-58

Comparison of cereal species according to the content of free asparagine and sugars as reactants in the Maillard reaction

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Cereals based food is one of the major source of heat induced contaminants in diet. Most of these compounds have extremely negative effect on human health. Acrylamide, as one of the food-borne toxicants that is formed as a result of the Maillard reaction, has been classified as a potential carcinogen to humans and genotoxic and neurotoxic to animals. Free asparagine (Asn) is a main precursor in formation of acrylamide in foods. The Asn alone may be converted thermally into acrylamide through decarboxylation and deamination reactions but the yield

of acrylamide from Asn is much higher when a carbonyl source is present. In order to determine genetic resource with reduced potential of acrylamide formation, the content of glucose, galactose, fructose, maltose, sucrose and free Asn were analyzed in a total of 42 genotypes of eight species (*Triticum aestivum*, *Triticum durum*, *Triticum dicoccon*, *Triticum spelta*, *Secale cereale*, *Hordeum vulgare* (var. *nudum*), *Avena sativa* (var. *nudum*) and *Zea mays*) grown at same location. According to our hypothesis, the genetic and agronomic approaches can be taken to reduce processing contaminants forming potential of cereal grains.

Our results provided the evidence of differences in content of sugars and Asn between and within seven species of small cereal grains. The average content of Asn in widely used species of cereals was found to be between 426 ± 144 and 1179 ± 359 mg/kg. The species had the mean Asn content with the following descending order: rye > hull-less oat > hull-less barley > durum wheat > bread wheat. The highest Asn content in rye and hull-less oat genotypes can potentially contribute to their very high acrylamide formation capacity. On average, as the main bread cereal, genotypes of *T. aestivum* var. *lutescens* species had the content of total reducing sugars and free Asn lower by 1.7- and 2.7-fold respectively, than rye genotypes. Sweet maize genotypes contained significantly higher amounts of all investigated sugars, except maltose, than other type of maize, and the lowest content of asparagine. Although the majority of pigment compounds of yellow, red and blue maize are glycosides, they were not in correlation with sugars content. PC analysis showed a high positive correlation between mono-reducing sugars and asparagine of bread wheat, durum wheat and hull-less barley and that the breeding can be simultaneously directed to a low content of both reactants.

PO-59

Colored maize and wheat as widely grown natural sources of anthocyanins

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Although almost 1000 anthocyanins have been identified in nature, the use of numerous potential plants as commercial sources of anthocyanin-based colorants is limited by availability of raw material, as well as economic considerations. Anthocyanins add not only colors to the food, but also potential health benefits to consumers because of their antioxidant properties. Health benefits associated with anthocyanin include treatment of various blood circulation disorders and neurodegenerative damages, anti-cancerous and anti-inflammatory properties, as well as controlling diabetes. It has been established that differences in anthocyanins structure i.e. in hydroxylation, methoxylation, glycosylation and acylation patterns of anthocyanins had a critical impact on its color and free radical scavenging capacity, and, in this connection, on their health-beneficial effects expression in human body. Colored grains hold promise as functional food colorants

given that cereal processing generates a large amount of anthocyanins-rich by-products at low cost. Therefore, the aim was to determine the composition and content of anthocyanins in grains of blue popping maize, deep purple maize and purple wheat.

According to our study, the content of total anthocyanins varied significantly between colored grains. Deep purple maize had the highest content of total anthocyanins as much as 4988.90 mg CGE/kg d.m. In comparison with the blue popping maize and purple wheat, deep purple maize had 5.5 and 54.4 times higher content of total anthocyanins, respectively. Ten anthocyanins were identified in blue popping maize, of which two are isomers of cyanidin-3-(malonylglucoside) and three of cyanidin-3-(dimalonyl- β -glucoside). In the deep purple maize, and purple wheat seven and nine anthocyanins have been identified, respectively. In its acylated and non-acylated form, cyanidin 3-glucoside was the dominant anthocyanins in the grain of all investigated genotypes. The content of cyanidin derivatives acylated with malonic acid was higher by 13 and 3.5 times in relation to the content of its glucosidic form in popping maize and wheat, respectively. Vice versa was observed in the grain of deep purple maize. The content of cyanidin 3-glucoside (Cy-3-Glu) and cyanidin 3,6-malonylglucoside (Cy-3,6-MalGlu) in deep purple maize was 1037.2 and 423.1 μ g/g, respectively. It can be concluded that rich in anthocyanins, colored grains could be used as functional food ingredient that provide health benefits to a large part of human world's population.

Authors' Index

A

Abraham, Aiman 27,
Aguzzoni, Agnese 39,
Akagi, Hirokatsu 71,
Al Iskandarani, Mohamad 71,
Amato, Elvio 28,
Arčon, Iztok 44,
Audi-Miro, Carme 63,

B

Bajc, Zlatka 70,
Baldini, Mario 53,
Ban, Dean 45,
Barceló, Juan 44,
Barre, Julien 38,
Bartella, Lucia 74, 46,
Basić, Zorica 77,
Bassi, Michele 39,
Bauer, Aleksandra 54,
Benedik, Ljudmila 45, 68,
Beone, Gian M. 67,
Bérail, Sylvain 38, 40,
Bergamaschi, Luigi 57,
Bergant, Janez 68,
Berisha, Sabina 73,
Bernabò, Nicola 71,
Bertuzzi, Terenzio 67, 69, 69,
Bešter, Erika 48,
Bizjak Bat, Karmen 77,
Blaznik, Urška 42,
Bogožalec Košir, Alexandra 42,
Boito, Marta 57,
Bolea-Fernandez, Eduardo 29,
Bontempo, Luana 27, 53, 56,
Brodie, Christopher 57,
Bučar-Miklavčič, Milena 48,
Butinar, Bojan 48,

C

Calaprice, Chiara 28,
Calvi, Marco 53,
Camin, Federica 40, 27, 54, 53, 31, 62, 56,
Cannavan, Andrew 27,
Carrey, Raul 63, 63,
Causon, Tim 47,
Cellier, Robin 38,
Chang, Ni-Na 58,
Chouhan, Raghuraj S. 73,
Christine, Oppen 39,
Ciglarič, Renata 70,
Cillero-Pastor, Berta 74,
Correia, Manuel 72,
Covaci, Adrian 66, 28,
Cuevas, Francisco 56,
Cuykx, Matthias 28,

Č

Černe, Marko 45,
Červek, Matjaž 46,

Ć

Ćirić, Andrija R. 65, 65, 59, 27,
Ćirić, Ivanka 58,

D

Dabić Zagorac, Dragana 58,
Dabić Zagorac, Dragana 58,
Debeljak, Marta 44,
Devoille, Laurent 72,
Di Donna, Leonardo 74, 46,
Diesner, Melanie 39,
Dodig, Dejan 77, 78,
Dolenc, Jožica 70,
Donard, Olivier F.X. 40, 38,
Drame, Anja 74,
Dražić, Goran 46,
Drinčić, Ana 40,
Durbiano, Francesca 69,

E

Eftimov, Tome 76, 32,
Elbashir, Hind 46,
Eler, Klemen 77,
Epova, Ekaterina N. 38, 40,
Erjavec, Karmen 49,

F

Fajon, Vesna 71, 76, 47, 41, 73,
Falnoga, Ingrid 41, 64, 41, 64,
Feldmann, Jörg 29,
Filipović, Vilim 45,
Focant, Francois 28,
Fontanella, Maria C. 67,
Fotirić Akšić, Milica 58,
France Štiglic, Alenka 41,
Frantzen, Sylvia 29,
Fujiyoshi, Lei 55,
Furdek Turk, Martina 40,

G

Gabriele, Iole 46,
Gašić, Uroš 58,
Golja, Viviana 46, 74,
Golubović, Jelena B. 37,
Goreta Ban, Smiljana 45,
Gorše, Leon 64,
Gradišar Centa, Urška 73,
Grčić, Nikola 78,
Grebenc, Tine 38, 38,
Griessler Bulc, Tjaša 66,
Gril, Mateja Naglič 62,
Grobler, Ana 64,
Guarnaccia, Paolo 53,
Gys, Celine 66,

H

Hajiboland, Roghieh 44,
Hamzić Gregorčič, Staša 54,
Hann, Stephan 39, 47,
Heath, Ester 37, 66, 66, 41,
Heath, David J. 37, 73, 41, 66,
Herrero-Martin, Sara 63, 63,
Herrmann, Gerald A. 32,
Hladnik, Jože 62,
Horvat, Milena 71, 41, 76, 64, 41, 47, 73, 43, 75,

I

Iacumin, Paola 57,
Ioannou Papayianni, Elena 77,
Ispirova, Gordana 76,

J

Jaćimović, Radojko 76, 45, 47,
Jagodic, Marta 75, 68, 64, 41, 60,
Jamnik, Polona 74,
Jandric, Zora 47,
Jeran, Zvonka 41,
Jerman, Ivan 73,
Joksović, Ljubinka G. 67,
Juchelka, Dieter 57,

K

Kanduč, Tjaša 60,
Katanić, Jelena 72, 70,
Kavčič, Anja 44,
Kavčič, Špela 68,
Kejžar, Jan 68,
Kelemen, Mitja 44,
Kelly, Simon 31, 27,
Klopčič, Marija 49,
Kobal, Alfred B. 41,
Kočar, Drago 55,
Kocman, David 41,
Kodre, Alojz 44,
Kokalj, Doris 62,
Kokkinofa, Rebekka 77,
Kononenko, Lijana 41,
Koron, Darinka 62,
Korošec, Mojca 61,
Koroušić Seljak, Barbara 76, 49,
Kos Skubic, Mira 49,
Kos Skubic, Mira 49,
Košir, Iztok J. 37, 37,
Kosjek, Tina 66, 66,
Kotnik, Jože 76,
Kovač, Nives 30,
Kovač, Marinka 64,
Kovačič, Ana 66, 66,
Kracht, Oliver 57,
Krajnc, Bor 38, 54,
Krsnik, Mladen 41,

Krupp, Eva 29,
Kušar, Anita 49,
Kvartuh, Rok 64,

L

Larcher, Roberto 56,
Latos-Brozio, Malgorzata 72,
Lobnik, Aleksandra 45,
Loeschner, Katrin 72, 29,
Lojen, Sonja 53,
Lojovic, Maja 54,
López-Sanz, Sara 72,
Lu, Jung-Tai 58,

M

Maage, Amund 29,
Mahne Opatić, Anja 53,
Major, Nikola 45,
Makuc, Damjan 61,
Mallia, Silvia 75,
Malus, Mirna 64,
Marc, Janja 41,
Markelj, Jernej 41,
Marosanovic, Biljana 54,
Martin, Maria 67,
Masek, Anna 72,
Masten, Jasmina 74, 60,
Mastnak, Tinkara 45,
Mazej, Darja 41, 41, 43, 75,
Mazzotti, Fabio 46, 74,
McGreevy, Steven 55,
Meglič, Vladimir 62,
Meland, Mekjell 58,
Mihailović, Vladimir B. 72, 70, 67,
Mihailović, Nevena R. 67,
Milačić, Radmila 43,
Milavec, Mojca 48,
Milivojević, Jasminka 58,
Močnik, Andrejka 70,
Mohren, Ronny 74,
Mokh, Samia 71,
Montenegro, Jose Carlos 56,
Moreno-Rojas, Jose Manuel 56,
Mounier, Lionnel 37,
Moussa, Fadl 71,
Mozetič Vodopivec, Branka 77,
Mrzlikar, Miha 41,
Mulazzi, Annalisa 69, 69,
Mulič, Ines 77,

N

Natić, Maja 58,
Natić, Maja 58,
Nečemer, Marijan 60, 68, 62, 37, 53,
59, 54,
Nedeljko, Polonca 45,
Nedić, Zoran 72,
Noireaux, Johanna 72,
Novak, Saša 74, 46,
Novotnik, Breda 43,

O

Ocvirk, Miha 37,

Ogrinc, Nives 61, 53, 62, 65, 60, 62,
65, 27, 57, 37, 38, 75, 59, 38, 54, 31,
77, 62, 74,
Osredkar, Joško 41,

P

Palčić, Igor 45,
Parareda-Sala, Joan 63,
Pasković, Igor 45,
Pavlin, Majda 73,
Pavlin, Anže 55,
Peeters, Kelly 43,
Pelicon, Primož 44,
Perini, Matteo 27, 62, 40,
Perković, Josipa 45,
Perrin, Sharona 75,
Pflieger, Marilynne 66,
Pianezze, Silvia 62,
Pignotti, Emanuela 39,
Pivk Kupirovič, Urška 49,
Plavec, Janez 61,
Poklar Ulrih, Natasa 30,
Poma, Giulia 28,
Pongrac, Paula 44,
Poschenrieder, Charlotte 44,
Potočnik, Doris 75, 27, 65,
Pravst, Igor 49,
Prohaska, Thomas 39, 47,
Prosen, Helena 41,
Prosenc, Franja 66,
Prpić, Igor 64, 41,
Puig, Roget 63,

R

Radke, Jens 57,
Rastelli, Silvia 69,
Regvar, Marjana 44,
Remškar, Maja 73,
Resnik Poljašević, Zala 64,
Robatscher, Peter 39,
Rolla, Umberto 67,
Romani, Marco 67,
Romić, Marija 45,
Rosell, Monica 63, 63,
Rossi, Andrea Mario 69,
Rossi, Filippo 69,
Rozenich, Rainer 27,
Rua-Ibarz, Ana 29,
Runkel, Agneta 43,
Rupprecht, Christoph 55,

Š

Šala, Martin 55,
Ščančar, Janez 43,
Škrjanc, Monika 61,
Škufca, David 66,
Šlejkovec, Zdenka 41, 41, 64,
Špirić, Zdravko 64, 41,
Štursa, Václav 47,

S

Salgot-Nadal, Valeri 63,
Sanden, Monica 29,

Scandellari, Francesca 39,
Schimmelmann, Arndt 31,
Sešek-Briški, Alenka 41, 41,
Sindona, Giovanni 46, 74,
Sinkovič, Lovro 62,
Slemc, Laura 61,
Smole Možina, Sonja 73,
Snoj Tratnik, Janja 75, 41, 43, 64, 41,
Soler, Albert 63, 63,
Spigno, Giorgia 69,
Srečković, Nikola Z. 70, 67, 72,
Sređević, Milica 58,
Stajenko, Anja 41, 41,
Stenni, Barbara 53,
Sterniša, Meta 73,
Stopar, Matej 62,
Strojniki, Lidija 38, 40,
Strojniki, Lidija 65, 62, 38, 59, 54, 62,
60, 27,
Sušin, Janez 68,
Szpunar, Joanna 28,

T

Tagliavini, Massimo 39,
Tayasu, Ichiro 55,
Tchaikovsky, Anastasiya 47,
Tchaikovsky, Anastasiya 39,
Tirler, Werner 39,
Titan, Primož 77, 78,
Tolrà, Roser 44,
Trdin, Ajda 41, 64,
Trdin, Miha 68,
Turetta, Clara 53,
Tuthorn, Mario 57,

U

Ule, Anita 49,
Unuk, Tina 38,
Užila, Zoran 45,

V

van Elteren, Johannes Teun 44,

V

Vančetović, Jelena 78,
Vanhaecke, Frank 29,
Vavpetič, Primož 44,
Vidmar, Janja 72,
Vidrih, Rajko 53,
Vogel-Mikuš, Katarina 60, 44, 54, 44,
Vreča, Polona 60, 47,
Vrhovsek, Urska 31,

W

Wan, Nai-Jung 58, 59,
Wanless, Dave 37,
Weber, Nika 62,

Y

Yarnes, Chris 54,

Ž

Ženko, Bernard 59,
Žigon, Dušan 43,

Žilić, Sladjana 77, 78,

Živković, Igor 76, 73,

Žnidaršič Pongrac, Vida 68,

Z

Ziller, Luca 56,

Zitek, Andreas 47,

Zitek, Andreas 39,

Zlatič, Emil 62,

Zoani, Claudia 31,

Zuliani, Tea 47, 53, 40, 60, 43,